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THE INCORPORATION OF DIRECT WITH PROTECTIVE INSECTICIDES AND FUNGICIDES

II. THE EFFECTS OF SPRAY SUPPLEMENTS ON THE RETENTION AND TENACITY OF PROTECTIVE DEPOSITS

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INTRODUCTION.

THE field efficiency of protective spray materials, such as Bordeaux mixture, lead arsenate and lime sulphur, which function through the formation of a deposit which protects the foliage from disease organisms or which limits the depredations of insect pests, is partly determined by the amount of deposit which persists upon the foliage throughout the period of attack. For convenience, the amount of deposit at any given time may be termed the spray residue. It is possible, by the addition of certain types of spray supplement to increase, in a variety of ways, the spray residue, such supplements being known by the general term "stickers". The purpose of the investigations to be described below is the examination of the action of such stickers and, in particular, the effects of wetting agents, added to combined washes to promote the efficiency of the direct insecticide or fungicide, on the spray residue of the protective constituent.

The practical problem is, however, not entirely solved by the investigation of the effect of the supplement upon spray residue for, with most protectants, the spray deposit is not primarily the active insecticide or fungicide. With lead arsenate, for example, the actual poisoning of the insect is probably due to the solution of part of the arsenate within the insect's intestine. The efficiency of the arsenical deposit is therefore conditioned by the relative amount of arsenate which is thus rendered soluble. Similarly, the constituent of the Bordeaux deposit which actively prevents the establishment of fungal infection is generally considered to be a soluble copper compound, derived by various agencies from the spray residue. The field performance of a protective spray material will therefore be dependent not only on factors associated with residue but also on those which determine the ease of formation of the active insecticide or fungicide. This latter property may conveniently be termed the availability of the residue.

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The quantity of spray residue will, in turn, be determined by at least two factors, first the actual amount of protectant retained on the foliage at spray application and second the ability of the initial deposit to withstand the various agencies which tend to its removal. The former quantity is termed the initial retention and has been the subject of investigations described in Part I of this series (1). The second factor, the resistance of the spray deposit to weathering, is termed the tenacity* of the deposit. In addition it is probable that qualities of the spray residue such as the uniformity of deposit, or " coverage ", will influence protective efficiency. This influence will, however, be exerted through the availability factors, in so far as the surface area of the deposit exposed to the action of availability agents is increased, and through tenacity in so far as uneven distribution will increase the effect of weathering agencies. The plan of the work to be described in the present paper is, accordingly, the laboratory investigation of the influence of supplements upon the initial retention and tenacity of protective spray materials applied in the form of suspensions, followed by an examination of the applicability of the laboratory conclusions to field conditions.

LABORATORY TRIALS.

EXPERIMENTAL METHODS.

(1) *Suspensions used.*—In selecting a substance suitable for use as the solid phase of the suspensions, the primary requirements were, (1), a compound the analytical determination of which in small amounts is simple, accurate and rapid, and (2), a compound which could be prepared when required as a dispersion of standard physical properties, in particular, particle size and degree of flocculation. The first requirement would be met by an insoluble copper compound and it was found that a suspension of cuprous iodide of suitable rate of sedimentation could be obtained by precipitation from saturated cupric sulphate solution on adding a slight excess of potassium iodide solution, sulphur dioxide being passed through to avoid the formation of free iodine. The cuprous iodide was washed free from excess iodide and potassium sulphate by sedimentation. After ten washings, the volume was adjusted, the spray supplement being added at this stage, to give a suspension of 1% weight cuprous iodide.

In later work, when the amount of suspension required could be foretold, a commercial red cuprous oxide was used. This preparation, containing

* The authors are indebted to Mr. R. W. Marsh for the suggestion of tenacity to define this quality. The expressions " initial retention " and " tenacity " have been adopted in favour of " initial adhesiveness " and " adherence " recently employed by Magie and Horsfall (3) for these two factors. It was considered that the latter terms suggest mechanical sticking, which does not appear to be involved in the determination of initial retention and which may not always be the mechanism responsible for tenacity. Retention and tenacity are terms which imply no hypothesis of the cause of the phenomena.

97% Cu_2O produced by an electrolytic process, was relatively free from the agglomerates present in the cuprous iodide. The particle size was such that by gentle agitation the spray could be kept in uniform dispersion.

(2) *Surfaces used.*—The artificial surfaces employed were glass coated with either paraffin wax, cellulose acetate or cellulose nitrate, particulars of which are given in Part I of this series. An additional surface to be used was of glass coated with a thermal-hardening glyptal resin. The glass plates were immersed in a 2·5% solution of the resin in Cellosolve (ethylene glycol mono-ethyl ether) and stoved at 120-30° C. for one hour.

(3) *The estimation of Initial Retention.*—Working with simple solutions, Evans and Martin showed that the maximum quantity of spray of wetting properties of the range under examination which could be carried by a vertical surface held at right angles to the direction of the spray is identical with the quantity applied at the point immediately prior to run-off or drain-off. The amount may therefore be determined, using the atomiser and pendulum method of application previously described, by multiplying the volume applied per square inch per exposure by the number of exposures. Before this method could be applied to the estimation of the initial retention of solid particles from a suspension, it was necessary to establish that there is no preferential retention of the solid phase in the system under investigation. For this purpose, paraffin and cellulose acetate surfaces were sprayed with 1% aqueous suspensions of cuprous iodide, the volume of spray applied being regulated by the number of swings of the pendulum and the aperture between the surface and the atomiser. To delimit the area of surface exposed to the spray it was covered by a glass plate with an aperture one inch square. The amount of copper retained upon the square inch of·exposed surface was determined by solution in warm dilute

TABLE I.

Retention of 1% Cuprous Iodide Suspensions.

Paraffin wax surface.			Cellulose acetate surface.		
No. of exposures.	mg. Cu.		No. of exposures.	mg. Cu.	
	Applied.	Retained.		Applied.	Retained.
50	0·49	0·49	50	0·51	0·51
76	0·81	0·81	55	0·57	0·57
92	0·95	0·74	57	0·59	0·48
116	1·19	0·68	76	0·78	0·43
			90	0·92	0·40
	Run off at 78 exposures.		100	1·03	0·41
				Run off at 56 exposures.	

nitric acid. After organic matter had been destroyed by evaporation with sulphuric acid on a sand bath, the copper was estimated colorimetrically by the potassium ferrocyanide method used by Martin (4).

Typical results, quoted in Table I, show that there is no preferential retention of the solid phase and the initial retention can be calculated from the volume of spray necessary to cause incipient run-off or drain-off.

(4) *The Estimation of Tenacity*.—It appears, on *a priori* grounds, impossible to deduce an absolute figure for tenacity, for the persistence of the solid phase remaining upon the surface after a given leaching treatment will be dependent upon the severity of that treatment, e.g. the amount of wash water applied and the force of application. The procedure adopted was accordingly empirical in character. The surfaces were sprayed with an amount of suspension never exceeding that required for run-off or drain-off. After drying for twenty-four hours at room temperatures the plate was refixed in the same position as when sprayed, the pendulum removed, and the deposit drenched with distilled water from the atomiser for one minute at an air pressure of 1·5 atmospheres. The deposit still remaining upon the surface was washed off with nitric acid and the amount of copper present determined by the method given above. The percentage still remaining of the total amount applied was termed the tenacity.

Because the retention of copper and the amounts of copper remaining after this leaching treatment were small, the area of the cellulose nitrate and glyptal resin surfaces exposed to the original spray was increased. The glass screen used in such cases had an aperture 4·5 × 3·0 cm. (=2·1 sq. inches) instead of 1 square inch.

EXPERIMENTAL RESULTS.

(1) *Analytical Data*.

Of the wide range of materials of potential value as spray supplements, a selection was made on the basis of the structural classification outlined by Evans and Martin (1). Of the long chain aliphatic derivatives, three were chosen in which the long chain is in the anion of the active constituent, viz. Sulphonated Lorol and C32, being of the fatty alcohol sulphate group, and Igepon T, being a fatty acid derivative. Of the derivatives of which the long chain is contained in the kation, Sapamine MS and C30 were selected, whilst, of the un-ionized surface active long chain derivatives, a water-soluble polyglycerol ester (U75) was employed.

Agral 2 and sulphite lye were selected of the spray supplements containing active constituents of cyclic structure, and of the miscellaneous unclassified "spreaders", gelatine, casein, saponin, a water-soluble methyl cellulose and a water-soluble synthetic resin were used.

The transition from simple solutions to suspensions enabled a number of supplements incompletely soluble in water to be used, e.g. lime casein, which has for long been employed in practice in combination with protectant spray materials. Trials were also made of various clays such as Fuller's Earth and bentonite, which have been suggested as spray supplements.

The value of glyceride oils as stickers for Bordeaux mixture was established by Martin (4) and, in America, petroleum oil emulsions are frequently employed as supplements for arsenical sprays applied against codling moth. Although the factors influencing spray retention in spray systems of two non-miscible liquids and a solid have not yet been examined in the laboratory, it was considered advisable to include oil emulsions in the present tests. Two such emulsions were used, and preliminary estimates of the effect of hydrocarbon oil emulsions upon the initial retention and tenacity of cuprous iodide and oxide were made in the laboratory using the method employed for simple suspensions.

Since many of the products mentioned above were from the same bulk sample as that used by Evans and Martin, it is unnecessary to give, in full, the analytical details on which the estimates of content of active constituents were based. Brief particulars of the character and amount of the active ingredients of each sample are as follows:—

Sulphonated Lorol.—This contains approximately 45·7% sodium "lorol" sulphate, "lorol" being a mixture of fatty alcohols in which dodecyl (lauryl) alcohol, $CH_3(CH_2)_{10}CH_2OH$, predominates; the chief concomitant material is the sodium salt of a sulphonated hydrogenated cyclic hydrocarbon.

C32.—This product, stated by the manufacturers to be a powder preparation of the product C72 (1), yielded, on acid hydrolysis, 27·5% ether-soluble fatty alcohol and 4·09% sulphur in the form of sulphate. The content of cetyl sodium sulphate may therefore be assessed at 39-40%. No indications were obtained of the presence of true sulphonates, as in C72. As the ash amounted only to 11·7% the concomitant material appears to be a water-soluble organic derivative.

Igepon T.—The figures quoted by Evans and Martin indicate that the sample contains about 40% taurine-oleic acid condensation product, the concomitant materials being mainly sodium chloride and sodium phosphate.

Sapamine MS.—The solution contains 0·59 gm. nitrogen per 100 ml. and gave, on prolonged acid hydrolysis, 4·8 gm. fatty acid (neutralization value 198) per 100 ml. These figures suggest that the preparation contains from 7·5-9% active constituent which is probably a quaternary ammonium sulphate of Sapamine Base, a condensation product of oleic acid (neutralization value 199) and a substituted ethylene diamine (Hartmann and Kagi, 2), the diluent being water.

C30.—This powder, described by the makers as a long chain alkyl quaternary ammonium bromide, gave 23·6% bromine and only 2·8% ash. It is therefore probable that the content of active constituent approaches 100%.

U75.—This product, of the consistency and appearance of soft soap, was described by the manufacturers as a polyglycerol ester containing about 66% active constituent, the remainder being water.

Agral 2.—For reasons given by Evans and Martin, this product was considered to contain approximately 75% alkylated naphthalene sulphonates as the active constituents.

Sulphite Lye.—Olive and Partington 60° Tw. syrup.

Casein.—A commercial Kahlbaum sample was mixed, before use, with certain proportions of hydrated lime. In addition, a soluble casein (Hopkin and Williams), probably containing the sodium salts of casein degradation products, was used.

Gelatine.—Coignet Extra.

Methyl Cellulose.—Purchased, as a water-soluble product, through a wholesale chemist.

C33.—A white powder, described by the makers as a water-soluble synthetic resin. It proved incompletely soluble in water and was therefore used in but few tests.

Fuller's Earth.—From laboratory stock, purchased through wholesale chemists.

Bentonite.—Both the ordinary commercial bentonite and the grade known as Wil-kinite, selected for detergent and emulsification purposes, were employed.

Cottonseed Oil Emulsion.—Two volumes of a cheap grade of edible cottonseed oil were emulsified in a laboratory mill with one volume of 20% sulphite lye (60° Tw.).

Petroleum Oil Emulsion.—The emulsion was prepared in the same manner as the cottonseed oil emulsion but, instead of the glyceride oil, two volumes of a highly refined (Grade G, 5) petroleum oil were used.

(2) *Initial Retention and Tenacity.*

The results of the systematic survey of spray supplements are assembled in Table II, the solid phase of the suspension being cuprous iodide and the surface paraffin wax. The tenacity figures are expressed as the mean percentage of six or seven replications and the standard error of the mean. Although theoretically a tenacity greater than 100% is impossible, experimental error is, in one case, responsible for a figure exceeding this limit. With gelatine and lime casein, the spray deposit was left as a film which was visibly unaffected by the leaching treatment.

TABLE II.

The Retention and Tenacity of Cuprous Iodide on a Paraffin Wax Surface.

Spray Supplement.	Conc. %	Initial Retention mg. Cu_2I_2/sq. inch.	Tenacity = % initial retention surviving leaching.
Sulphonated Lorol	0·5	1·39	25 ± 4·6
	0·1	1·61	42 ± 3·1
	0·05	1·75	59 ± 2·1
	0·025	1·84	65 ± 5·9
C32	0·5	0·67	45 ± 6·5
	0·05	1·39	88 ± 6·5
Igepon T	0·5	(0·63)	40 ± 7·3
	0·1	(0·85)	64 ± 1·9
	0·05	0·97	90 ± 1·9
	0·025	1·06	100 ± 4·1
Sapamine MS	0·5	0·89	71 ± 2·9
	0·1	0·97	87 ± 2·4
	0·05	1·12	95 ± 5·1
C30	0·5	0·87	95 ± 3·5
	0·1	0·71	100 ± 6·3
U75	0·5	0·50	49 ± 3·9
	0·1	0·69	75 ± 5·4
	0·05	0·75	81 ± 9·4
	0·025	0·94	85 ± 8·6
Agral 2	0·5	1·41	35 ± 2·7
	0·1	1·90	52 ± 2·2
	0·05	2·02	77 ± 3·3
	0·025	2·01	95 ± 5·0
Sulphite Lye	1·0	3·12	10 ± 0·5
	0·5	3·25	63 ± 3·3
	0·1	1·97	65 ± 5·2
	0·05	(1·99)	82 ± 8·2
Gelatine	0·5	1·59	100 —
	0·1	1·34	100 —
	0·05	1·65	100 —
Lime Casein	0·5	0·91	100 —
	0·1	1·45	100 —
	0·05	1·72	100 —
	0·025	2·34	100 —
Saponin	0·5	0·48	68 ± 4·5
	0·1	(0·54)	95 ± 9·2
	0·05	0·68	104 ± 5·5
	0·025	(3·10)	100 ±10·0
Methyl Cellulose	0·5	0·86	27 ± 5·7
	0·1	0·76	77 ± 2·5
	0·05	0·80	78 ± 2·5
	0·025	0·80	95 ± 3·5
Nil	—	2·10	100 ± 3·0

TABLE III.

The Retention and Tenacity of Cuprous Iodide on a Cellulose Nitrate Surface.

Spray Supplement.	Conc. %	Initial Retention mg. Cu_2I_2/sq. inch.	Tenacity = % initial retention surviving leaching.
Sulphonated Lorol	0.2	0.30	5 ± 0.5
	0.05	0.45	14 ± 2.3
	0.025	0.73	53 ± 2.0
C32	0.2	0.29	39 ± 1.7
	0.05	0.32	70 ± 5.0
	0.025	0.55	84 ± 2.9
Igepon T	0.2	0.22	52 ± 4.0
	0.05	0.31	57 ± 0.9
	0.025	0.42	68 ± 4.6
Agral 2	0.2	0.35	11 ± 2.2
	0.05	0.95	61 ± 6.7
	0.025	1.42	73 ± 5.4
Sulphite Lye	0.5	0.46	2 ± 0.8
	0.1	1.11	3 ± 0.9
	0.05	(1.44)	7 ± 2.9
	0.025	(1.63)	9 ± 2.8
Gelatine	0.05	0.39	88 ± 2.8
	0.025	0.47	76 ± 4.7
	0.01	(1.2)	79 ± 5.9
Casein (soluble)	0.1	—	90 ± 0.9
	0.05	—	91 ± 6.7
	0.025	—	90 ± 4.2
	0.01	—	76 ± 4.3
Methyl cellulose	0.05	0.51	23 ± 3.8
	0.025	0.47	9 ± 2.5
	0.01	(0.50)	29 ± 1.2
	0.005	0.63	50 ± 1.4
C33	0.2	—	90 ± 3.1
	0.025	—	97 ± 6.1
Fuller's earth	1.0	1.91	7 ± 2.4
Bentonite	1.0	1.91	45 ± 6.3
Wil-kinite	1.0	1.91	17 ± 4.2
Nil	—	1.91	28 ± 3.0
Lime (hydrated)	1.0	1.91	23 ± 1.9
Petroleum oil emulsion	1.0	1.97	78 ± 2.7

Table III contains the results obtained on the cellulose nitrate surface whilst Table IV shows the results with the glyptal resin surface. In the last-named series the solid phase of the suspension was red cuprous oxide.

TABLE IV.

The Retention and Tenacity of Cuprous Oxide on the Glyptal Resin Surface.

Spray Supplement.	Conc. %	Initial Retention mg. Cu_2O/sq. inch.	Tenacity %
Sulphite Lye	0·75	0·73	65 ± 3·0
Methyl cellulose	0·005	0·38	86 ± 2·9
Sulphonated Lorol	0·05	0·27	87 ± 3·3
Lime	1·0	0·69	87 ± 2·9
Agral 2	0·05	0·33	91 ± 3·0
Nil	—	0·92	92 ± 3·8
Gelatine	0·1	0·29	100 ± 1·9
Petroleum oil emulsion	1·0	0·68	101 ± 2·1
Lime Casein (Lime : Cas. 7 : 1)	0·25	0·26	101 ± 3·0

TABLE V.

Number of Exposures necessary to give Excess of Spray.

Surface.	Spray.		Number of exposures.	Mean.
Paraffin	Water	Alone	80 ; 72 ; 77 ; 94 ; 87 ; 78	81·0
		+Cu_2I_2	90 ; 72 ; 85 ; 80 ; 77	80·8
,,	0·5% Sulph. Lorol	Alone	44 ; 46 ; 45	45·0
		+Cu_2I_2	45 ; 48 ; 48 ; 46	46·8
,,	0·5% Sapamine	Alone	29 , 30 ; 29	29·3
		+Cu_2I_2	28 ; 30 ; 30	29·3
,,	1·0% Sulphite Lye	Alone	85 ; 88 ; 84 ; 84	85·2
		+Cu_2I_2	84 ; 86 ; 83 ; 87	85·0
,,	Lime Casein (0·05% casein)	Alone	18 ; 20 ; 19	19·0
		+Cu_2O	19 ; 19 ; 20	19·3
Cellulose Nitrate	0·5% Methyl cellulose	Alone	13 ; 13 ; 13	13·0
		+Cu_2I_2	13 ; 13	13·0
Glyptal resin	0·005% Methyl cellulose	Alone	16 ; 16 ; 16	16·0
		+Cu_2O	16 ; 15 ; 16	15·7
,,	Lime Casein (0·05% casein)	Alone	12 ; 11 ; 12 ; 11	11·5
		+Cu_2O	12 ; 12 ; 11 ; 11	11·5

DISCUSSION.

Initial Retention.—In Part I of this series, Evans and Martin showed that the maximum amount of spray initially retained upon a plane surface is determined by wetting and spreading properties, markedly decreasing with increase of these properties. It was anticipated that the addition of the suspended particles might modify the wetting and spreading properties of the aqueous phase and thereby affect spray retention. This anticipation was not always realized and, in the majority of cases, the presence of the solid did not alter the volume of spray required to achieve run-off or drain-off. In Table V, for example, the number of exposures required to attain excess of spray with and without cuprous iodide and red cuprous oxide, respectively, are recorded.

In only a few of the systems examined did the presence of the solid affect initial retention. The results obtained with these systems are given in Table VI, in which the number of exposures required to give run-off are recorded and the weight of spray retained is calculated from the average retention figure for water on paraffin wax (=210 mg. per sq. inch).

TABLE VI.

Abnormal Retention at Run-off.

System.	Conc. of supplement %	No. of exposures to give run-off.		Retention mg./sq. inch.	
		Aq. sol.	Cu_2I_2 suspension.	Aq. sol.	Cu_2I_2 suspension.
Gelatine/ paraffin surface	0·5	80	50	255	159
	0·1	64	42	204	134
	0·05	116	52	369	165
	0·025	146	74	465	235
	0·000	86	66	(210)	(210)
Methyl cellulose/ paraffin surface	0·5	65	43	130	86
	0·1	53	38	106	76
	0·05	52	40	104	80
	0·025	53	40	106	80
	0·01	60	49	120	98
	0·005	69	59	138	118
	0·000	105	—	(210)	—
			Cu_2O suspension.		Cu_2O suspension.
Sulphite Lye/ Glyptal surface	0·75	24	20	118	98
Petroleum oil emulsion/ Glyptal surface	1·0	37	31	181	152

With the glyptal surface it was found that the addition of hydrated lime modified the degree of retention of water. Thus, the retention of water alone was 204 mg. per sq. inch, whereas that of the lime suspension was 152 mg. per sq. inch. No detailed examination of this phenomenon was made but in this case and in those in which the addition of cuprous iodide or cuprous oxide affected retention, it was observed that the addition of the solid particles visibly affected the wetting properties of the spray, for the droplets exhibited a lower contact angle than the simple aqueous solution. It may be noted that the effect was not observed upon the cellulose nitrate surface. Here the effect may have been masked by experimental error but the observation suggests that the nature of the solid surface should not be neglected in the subsequent investigation of the phenomenon.

The observation that, in the majority of systems, the addition of the solid phase was without influence upon initial retention enabled a simplification of the laboratory work, for it became necessary only to establish, at the highest concentration of supplement used, that the addition of the solid particles did not change the number of exposures required for run-off. If this proved to be the case, it could be assumed that the retentions of suspensions containing lower concentrations of the spray supplement were identical with those of the aqueous solution of the supplement. As this figure was known from previous work, the amount of copper retained upon the surface could be calculated. This amount is recorded in Tables II and III as mg. cuprous iodide per sq. inch.

In view of the close similarity between the retention of suspensions and the retention of the aqueous phase alone, further comment on the figures may be postponed until a more detailed examination of the correlations between retention and wetting and spreading properties has been made. It may, however, be noted that for those systems in which the presence of the solid particles has no influence upon retention, it follows that particle size, up to the maximum used in the tests, is not a factor influencing initial retention. This corollary is naturally not applicable to those surfaces encountered in practice on which the presence of hairs or fungal threads leads to an accumulation of solid through filtration. It does, however, indicate that particle size exerts its influence upon the efficiency of a protectant spray more through its effect upon coverage than upon initial retention.

Tenacity.—The systems under examination are of three components, the liquid, the solid particle and the surface. The interdependence of these three variables in affecting the tenacity of the spray deposit is well shown by cuprous iodide in water suspension. On the paraffin wax surface the spray deposit was unaffected by the leaching treatment, whereas, on the cellulose nitrate, only 28% survived. This difference is probably explained by the observation that,

on the cellulose nitrate surface, the cuprous iodide deposit shrank during drying, a process which no doubt renders the deposit less tenacious. Shrinkage did not occur either on the paraffin surface or with the more crystalline cuprous oxide particles. Moreover it was prevented by the addition of small amounts of supplements and, in such cases, even highly surface-active wetting-out agents in small amount improved tenacity, thereby functioning as true " stickers ". The only exceptions observed were Fuller's Earth and Wil-kinite, the deleterious effect of which on tenacity seems to be due to an enhancement of this colloidal shrinkage.

Where shrinkage was not a disturbing factor it was generally found that the addition of supplements of high surface activity decreased tenacity ; with these supplements (e.g. Sulphonated Lorol, C32, Igepon T, Sapamine MS, C30, U75, Agral 2 and Saponin) the reduction of tenacity became greater as the concentration of supplement was increased. As the results supply no indication that the molecular structure of the supplement influences the degree of reduction of tenacity, the probable explanation of this action is that the presence of a wetting agent in the spray residue renders it more easily wetted by the leaching water, resulting in a greater loss of the deposit. The relatively greater decrease in tenacity of cuprous iodide upon cellulose nitrate as compared with that on paraffin wax is in accordance with this hypothesis. Sulphite lye is outstanding in its deleterious effect upon tenacity, a property which may be ascribed to its exceptional powers as a dispersing and emulsifying agent. It is feasible to suppose that those properties which promote dispersion will function in the spray residue dispersing it in the leaching water and thereby reducing tenacity.

Of the supplements which were added primarily as " stickers ", e.g. gelatine, lime casein and methyl cellulose, the first two show a favourable effect on tenacity. All three supplements yield suspensions which, on paraffin wax, show the type of drain-off designated (a) by Evans and Martin, an indication of high wetting properties. The apparent improvement in wetting properties induced by the presence of solid particles in solutions of gelatine and methyl cellulose has already been illustrated by Table VI. On drying, however, the films are no longer readily wetted by the leaching water, and the removal of spray residue is therefore low. With methyl cellulose, which, even at the lowest concentrations used imparted good wetting properties to the suspensions, the low tenacity may be associated with its relatively greater solubility in the leaching water. On this hypothesis it may be concluded that for a spray supplement to exhibit good wetting and spreading properties and yet have no unfavourable influence upon tenacity it should, after application, dry to a deposit not easily wetted by water.

FIELD TRIALS.

In order to check the conclusions of the laboratory tests by field trials under practical conditions, it was decided to select a crop plant on which the efficiencies of the protectant sprays could simultaneously be determined by biological assay. Of the possible crop plants, potato was chosen as that most suitable, for the following reasons: (1), the efficiencies of the various sprays as protectants against Blight (*Phytophthora infestans*) could be compared, provided the disease appeared in the plots or in the unsprayed control plants; (2), attack by this fungus usually occurs after the rapid initial development of leaf surface has ceased and, accordingly, errors due to the presence of unsprayed new growth are reduced; (3), the potato is, by virtue of its low habit of growth, easy to spray, the foliage is easily sampled and the degree of Blight infestation relatively easy to record; (4), the acreage required for the trials is small; (5), the provision of a suitable supply of test material offers no difficulty.

EXPERIMENTAL METHODS.

(1) *Lay-out of Trial.*—The area available for the test was a plantation of pears interplanted within the rows with red currants and laid out in 1935. A double row of King Edward potatoes, a variety chosen because of its susceptibility to Blight, was planted between each pear row. Scotch seed was used to reduce the complications of disease recording and foliage sampling caused by virus diseases.

Each of the thirteen double rows was subdivided into lengths of ten paces, making 130 individual plots. The spray treatments were distributed so that each of the ten blocks, one block running at right angles to the direction of the rows, constituted a randomized block.

(2) *Sprays used.*—Of the thirteen spray treatments, only ten were concerned with the influence of spray supplements upon protective fungicidal properties. The protective fungicide used was a paste preparation of yellow cuprous oxide (35% Cu). To prevent sedimentation to an unworkable deposit during storage a minimum of protective colloid, 1·9% powdered sulphite lye, was incorporated in the preparation whilst, to overcome frothing during manufacture, a small amount (0·2%) of a proprietary anti-foaming agent was added.

The spray supplements tested were added to the cuprous oxide spray (containing 0·2% metallic copper) at the following rates:—

Treatment 6: Sulphonated Lorol, 8 oz. per 100 gal.
    ,,   5: Agral 2, 8 oz. per 100 gal.
    ,,   3: Sulphite lye, 6 pints syrup (60° Tw) per 100 gal.
            (=approx. 1%).

Treatment 10 : Gelatine, 1 lb. per 100 gal.

,, 　　2 : Lime casein, 10 lb. hydrated lime and 8 oz. casein per 100 gal.

,, 　　1 : Lime, 10 lb. hydrated lime per 100 gal.

,, 　　4 : Methyl cellulose, 0·8 oz. per 100 gal. (0·005%).

,, 　　8 : Cotton seed oil emulsion, 1 gal. (=0·67 gal. cotton seed oil and 0·33 gal. 20% sulphite lye) per 100 gal.

,, 　　9 : Petroleum oil emulsion, 1 gal. (=0·67 gal. oil and 0·33 gal. 20% sulphite lye) per 100 gal.

,, 　　7 : No added supplement.

Particulars of the various products used are given on pp. 5 and 6.

(3) *Spray application.*—To enable comparisons to be made between all treatments it was necessary to complete each application in one day. As one hundred widely-distributed plots had to be sprayed the most rapid method possible was required. Further, to eliminate variation in spray retention due to uneven application, each plot had to receive equal amounts of spray. Despite the apparent departure from practical spraying methods, the one selected was by means of watering cans, each plot receiving the contents of a 1 gal. can. In view of the relative ease with which potato foliage is wetted, it was considered improbable that the force of application, which might be of importance with a solid/liquid system exhibiting a high receding contact angle, would affect spray retention. Further, the emulsions, the stability of which might be enhanced by passage through spray pumps and nozzles, were initially of a high degree of dispersion and stability.

The first application was begun on July 22nd and treatments 3, 4, 5 and 8 were applied under perfect conditions. Early rain on July 23rd prevented prompt completion of the application, and soon after treatment 10 had been applied a slight shower fell before the spray had dried on the foliage. It was considered possible, but improbable, that the shower had affected spray residue. The second application was made under good conditions on August 7th.

(4) *Estimates of Spray Residue.*—For the estimation of the amount of copper protectant remaining on the foliage throughout the trial, periodic samplings of the foliage were made. Twenty leaflets were collected at random from each plot and the ten samples per treatment were mixed in the laboratory. From each leaflet, a disc of 1·325 cm. diameter was cut by means of a cork borer, care being taken to avoid the edges and tips of the leaflets. The four samples of fifty discs per treatment were ashed separately and the copper present was determined by the colorimetric ferrocyanide method.

(5) *Estimation of Blight Infestation.*—Previous but, as yet, unrecorded experience of the estimation of Blight infestation has indicated that a suitable

method is to assess the degree of prevalence of the disease on the foliage of each plot which is given an estimate based upon the following scale :—

0 = No Blight apparent.

2 = Blight lesions scarce.

4 =   ,,   ,,   occasional.

6 =   ,,   ,,   frequent.

8 =   ,,   ,,   ,,   with defoliation.

10 = Haulm completely defoliated.

The odd numbers were used for intermediate degrees of Blight infestation.

EXPERIMENTAL RESULTS.

(1) *Estimates of Spray Residue.*—Foliage sampling was carried out as soon as possible after each application in order that the figure might give a measure of initial retention. Unfortunately, frequent showers during and after the first application caused delay and sampling was not completed until July 25th. The second sampling was made on August 5th, just prior to the second application. Following that application no rain fell until after the third sampling was completed on August 11th. The fourth and final sampling was made on August 27th, by which time the disease had caused such serious defoliation that no further samples were taken, for it was feared that Blight might have preferentially attacked those leaflets carrying a small proportion of spray residue and thereby prevented true sampling.

TABLE VII.

Estimates of Copper Retention.

Treatment	1	2	3	4	5	6	7	8	9	10
Date of Sampling										
24 & 25.7.36	4·8	3·3	4·2	5·0	4·5	5·3	8·2	4·2	6·8	4·3
	7 8	3·0	4·5	5·5	4·0	4·8	5·2	6·4	6·1	2·7
	8·4	3·5	5·2	5·6	4·2	4·6	5·5	7·6	7·2	3·8
	7·4	3·7	5·6	4·2	—	4·2	6·2	6·6	6·7	3·8
5.8.36	4·5	2·4	2·3	2·8	3·6	3·5	3·2	3·6	4·6	2·4
	3·5	3·1	2·2	4·5	3·5	4·3	3·2	3·2	4·2	2·7
	4·7	2·5	2·0	2·1	3·0	2·3	3·2	4·8	5·4	2·3
	—	2·6	2·2	3·2	3·1	2·5	3·4	4·2	4·3	3·3
11.8.36	14·4	12·4	13·8	13·4	6·1	14·0	14·8	18·2	24·4	11·0
	20·0	10·4	15·2	16·4	8·5	12·0	14·0	21·2	27·8	13·8
	12·4	8·2	20·4	16·0	6·0	14·0	12·0	18·2	26·0	16·8
	16·8	14·0	18·0	12·0	8·0	10·0	15·6	20·2	27·6	12·8
27.8.36	12·8	6·6	8·0	9·6	6·1	7·0	10·3	16·4	24·2	8·8
	9·4	9·4	9·0	10·0	5·8	7·4	8·2	15·6	20·0	8·4
	9·4	8·4	9·4	9·8	7·6	7·8	9·8	20·6	27·4	10·0
	8·6	10·2	10·4	9·6	5·0	9·2	12·2	18·8	24·0	8·0

The analytical results are recorded in Table VII which gives the amount of copper expressed as mg. Cu $\times$ 10^{-1} per 50 leaf discs of diameter 1·325 cm., found in each sample. The analysis of variance of these results is summarized in Table IX.

TABLE VIII.

Estimates of Blight Infestation.

Treatment	1	2	3	4	5	6	7	8	9	10
Block I	2	2	2	1	2	3	2	3	2	2
	3	3	3	3	3	4	3	3	3	4
	4	4	5	4	5	5	4	4	5	5
	6	6	8	7	7	8	7	7	6	7
Block II	2	3	3	2	3	2	2	2	3	1
	3	4	4	4	4	3	4	3	4	4
	5	6	5	5	6	4	6	4	5	4
	8	8	8	7	8	8	8	7	6	7
Block III	1	3	2	2	2	3	3	1	2	2
	3	5	4	3	4	5	4	3	3	3
	4	6	5	5	5	6	6	3	4	5
	7	9	8	8	7	8	8	7	6	8
Block IV	2	1	2	2	2	3	3	3	1	3
	4	3	3	5	3	5	4	6	3	4
	6	4	4	3	6	6	6	7	4	6
	9	7	7	7	8	9	8	9	6	9
Block V	2	2	2	3	3	2	3	3	2	3
	3	3	3	4	4	4	5	5	4	5
	4	5	5	5	6	5	7	7	6	6
	7	7	8	8	8	8	9	8	8	9
Block VI	3	1	2	2	3	2	3	3	3	2
	4	4	4	4	5	4	5	4	4	4
	5	5	5	6	7	5	6	5	5	5
	7	9	8	8	9	8	8	8	7	8
Block VII	2	1	3	3	3	2	3	3	2	3
	4	4	4	5	5	4	5	4	4	5
	5	6	6	6	6	5	6	5	6	6
	7	9	8	9	9	8	9	8	8	9
Block VIII	2	3	2	2	3	3	2	2	3	4
	5	5	4	4	5	4	3	4	5	6
	6	6	5	6	7	5	5	6	7	7
	9	9	8	8	9	7	7	8	9	9
Block IX	2	2	2	3	4	2	3	3	3	3
	5	3	4	5	6	4	5	5	5	5
	6	4	4	7	7	6	7	7	6	6
	8	6	7	9	9	8	9	9	8	9
Block X	4	3	3	3	3	3	3	2	2	3
	6	5	4	5	4	4	5	4	3	5
	7	7	6	7	5	5	7	5	5	6
	9	9	9	10	7	7	10	7	7	9

(2) *Estimates of Blight Infestation.*—On July 22nd, prior to the first application, the degree of Blight infestation was uniform throughout the plots and was estimated as 1. The remaining estimates are given in Table VIII, the estimates for each plot made on July 28th, August 6th, 17th and 29th being recorded in this order vertically for each plot. On the final date, it was decided that, as the estimates on many plots approached the limit of 10, further estimates would not supply useful data. The estimates were submitted to statistical analysis with the results given in Table X. In both Tables IX and X the results received for the remaining three spray treatments are included.

TABLE IX.

Statistical Analysis of Estimates of Spray Residue.

	Degrees of Freedom.	Sum of Squares of Deviations.	Variance.
Treatments ..	12	1,914·85	159·57
Dates	3	5,054·88	—
Remainder	188	1,414·43	7·52

TABLE X.

Statistical Analysis of Estimates of Blight Infestation.

	Degrees of Freedom.	Sum of Squares of Deviations.	Variance.
Treatments (T) ..	12	38·0	3·167
Blocks (B)	9	87·0	—
Dates (D)	3	2,138·8	—
Interactions :			
T × B	108	188·0	—
B × D	27	8·7	—
T × D	36	10·4	—
Remainder	324	77·1	0·238

DISCUSSION.

For the discussion of the field results and their concordance with laboratory results, it is convenient to analyse the data on the basis proposed in the introductory section of the present paper. It is there suggested that protective efficiency is determined by two main sets of factors, the availability and the spray residue factors, the latter being the resultant of the factors influencing initial retention and those determining tenacity.

THE INFLUENCE OF AVAILABILITY FACTORS.

To separate the influence of these factors from the effects of spray residue upon the efficiency of the protectant, the degree of control as measured by the

average Blight estimate for each treatment may be compared with the spray residue on the assumption that the mean figure for copper deposit represents a summation of the amounts of copper present throughout the period of the test. This assumption implies that the mean copper retention figure represents the average amount of material capable of being rendered fungicidal and present on the foliage at each time when environmental conditions favour infection.

TABLE XI.

Comparison of Blight Infestation and Copper Residue.

Spray supplement.	Mean of 40 Blight estimates.	Spray residue.	Mean of copper estimates.	No. of estimates.
Petroleum oil	4·625	Petroleum oil	15·42	16
Sulphite lye	4·725	Cottonseed oil	11·86	16
Lime	4·775	Lime	9·32	15
Lime casein	4·800	Nil	8·44	16
Cottonseed oil ⎫		Sulphite lye	8·31	16
Sulphonated Lorol ⎬	4·925	Methyl cellulose	8·11	16
Methyl cellulose	5·000	Gelatine	7·18	16
Gelatine	5·275	Sulphonated Lorol	7·06	16
Agral 2	5·200	Lime casein	6·48	16
Nil	5·325	Agral 2	5·25	15
Significant Difference (P=0·05)	0·222	between means of 16 estimates		1·98
		between means of 15 and 16 estimates		2·02
		between means of 15 estimates		2·08

In Table XI, the various treatments are arranged in the first column in order of protective efficiency as judged by the average Blight estimate per treatment and, in the third column, in order of the average copper residue. A comparison of the two orders of merit reveals a high degree of correlation and, in the cases of non-agreement, it is possible to advance tentative explanations. The fungicidal efficiency of cuprous oxide in the absence of a spray supplement (the 0·01% sulphite lye derived from the preparation may be considered negligible) is lower than would be expected from the mean copper residue, an indication that the addition of the supplement has induced a more uniform distribution of the copper compound and, thereby, enhanced in a sense its availability. This effect might account for the improvement of protectant qualities conferred by sulphite lye, lime casein, Sulphonated Lorol and Agral 2. The relatively low position of the cotton seed oil spray in order of fungicidal efficiency as compared with its position in the third column suggests that cotton seed oil has in some way, perhaps by virtue of its small content of free fatty acid, affected availability in a manner not definitely shown by the petroleum oil. Further, as no reduction of fungicidal activity is observable when casein (as lime casein) or gelatine is added, it may be concluded that these proteins do not reduce the

availability of the copper fungicide, an observation of interest in connection with the hypothesis that the toxic action of copper compounds is associated with a combination with, or an adsorption by, the protein of the organism. If this were the mode of action of the copper fungicides, it would seem that the presence of extraneous protein would reduce activity, an effect not observed in the above trials.

THE INFLUENCE OF THE SUPPLEMENT ON INITIAL RETENTION.

Proceeding with the analysis of the copper residue estimates, the initial retention may be considered to be represented approximately by the copper estimates of the first foliage samplings after each spray application. Neither in the sample taken on July 24th/25th nor in that of August 11th does the figure supply a true measure of spray retention for, on the first occasion, rain intervened between spraying and sampling, whilst the figure obtained on the second date included spray residue from the first application. In Table XII, the various treatments are arranged in each column in the order of spray residue.

TABLE XII.

Initial Retention of Copper on Foliage.

First Application.			Second Application.	
Spray Supplement.	Mean copper estimate.	No. of estimates.	Spray Supplement.	Mean of four copper estimates.
Lime	7·10	4	Petroleum oil	26·25
Petroleum oil	6·70	4	Cottonseed oil	19·35
Nil	6·27	4	Sulphite lye	16·80
Cottonseed oil	6·20	4	Lime	15·90
Methyl cellulose	5·07	4	Methyl cellulose	14·45
Sulphite lye	4·87	4	Nil	14·10
Sulphonated Lorol	4·72	4	Gelatine	13·60
Agral 2	4·23	3	Sulphonated Lorol	12·50
Gelatine	3·65	4	Lime casein	11·25
Lime casein	3·37	4	Agral 2	7·15
Significant difference (P=0·05)	1·50 1·70	4 3 and 4		4·27

The large difference required for significance is doubtless responsible for many of the apparent inconsistencies in the two relative orders, but interesting points of agreement are shown. Noteworthy are the high positions of the oils and the low positions of Sulphonated Lorol and Agral 2, supplements of high surface activity. Lime casein is, in both columns, low as compared with lime, whereas gelatine is lower than might be expected by reason of its high viscosity

which, as Woodman (6) has stated, would cause an increase in the volume of spray retained by leaves in spraying.

No strict comparison of the field results and the laboratory results on initial retention can be made, for the latter represent the maxima which can be attained. Further, in the laboratory, the surfaces are held at right angles to the direction of the spray, under which conditions the total volume applied is retained until the maximum is reached. In the field the leaf surface is rarely, if ever, perpendicular to the direction of the spray and, under such conditions, only a fraction of the total spray applied is retained, the magnitude of the fraction being related, in a manner as yet uninvestigated, to the wetting and spreading properties of the spray on the foliage concerned. To illustrate this point, certain results obtained by Mr. B. Collie in this laboratory may be quoted. In these experiments the surface was tilted at 45° to the direction of the spray and the volume required to produce run-off was determined.

TABLE XIII.

Fraction of Spray retained on Inclined Surface.

	Paraffin Wax.				Cellulose Acetate.			
Conc. %	o	o·1	o·5	1·0	o	o·1	o·5	1·0
Igepon T	0·63	0·67	0·65	0·74	0·65	0·70	0·72	0·81
Agral 2	0·66	0·68	0·69	0·75	0·63	0·66	0·68	0·81
Sulphonated Lorol	0·6o	0·66	0·66	0·72	—	0·62	0·65	0·74
C32	0·60	0·64	0·63	0·71	0·64	0·65	0·66	0·76

In Table XIII, the volume of spray retained per unit area of tilted surface is expressed as a fraction of the actual amount applied per unit area of surface perpendicular to the axis of the spray cone. As the area exposed to the spray is increased by tilting, the theoretical ratio indicating complete retention is sin. 45° = 0·71. That this figure is sometimes exceeded is due, in part, to the nearer approach of part of the tilted surface to the spray nozzle. The figures show that, with water and the lower concentrations of supplements, part of the volume of spray applied is not retained. Consequently it may be assumed that of the first amounts of spray to reach the foliage, the proportion retained increases with the wetting and spreading properties of the spray. The maximum retention figure for each particular spray is therefore more rapidly attained with sprays of good wetting and spreading properties. Further, with surfaces held perpendicular to the spray, the initial retention figures are lower with liquid/solid systems exhibiting low contact angles. For comparison with field results in which no great excess of spray was applied, it is, for these reasons better to select laboratory results obtained on a readily wetted surface.

In Table XIV, the supplements are arranged in order of initial retention on paraffin wax, cellulose and glyptal resin surfaces, the concentration of each supplement being that used in the field trials, with the exception of lime casein (0·25% 1:4 mixture of hydrated lime) on the glyptal resin surface. The results show, as indicated in Table XIV, that the maxima are relatively lower on the more readily wetted cellulose nitrate surface than on the paraffin wax. For comparison with the field results given in Table XII, the cellulose nitrate surface, which incidentally approximates to potato foliage in relative ease of wetting, is selected. On this surface, the oil, no supplement, and lime occupy the high positions, whereas gelatine and lime casein are low, as in the field trials. The most marked exception is Agral 2 which gives a relatively greater initial retention in the laboratory than in the field. Taking into consideration the various factors limiting complete concordance between laboratory and field results, the degree of agreement is encouragingly high.

The Effect of the Supplement on Tenacity.

To represent the field estimates of tenacity, the amounts of residue for the second and fourth samples, expressed as percentages of the residue at the first and third samplings respectively, may be taken. These percentages have no absolute significance and the two sets of figures obtained from the two applications will bear no strict proportionality, for each set represents tenacity under different conditions of time and weathering. The figures can therefore be used only to place the various supplements in order of their favourable or unfavourable effect upon tenacity. These orders are given in Table XV in which are included the laboratory figures obtained with cuprous iodide suspensions on the cellulose nitrate surface and with cuprous oxide suspensions on the glyptal resin surface, the concentration of each supplement being that employed in the field trials. No laboratory figures are available for the cottonseed oil emulsion.

Noteworthy among the results shown in Table XV is the favourable effect of lime casein and gelatine upon tenacity, an observation, in the latter case, in agreement with Woodman's (6) prediction that gelatine, being relatively insoluble in cold water, would render the spray deposit less liable to be washed off by rain. The effect of lime casein is in marked contradistinction to that of lime, with which the results are inconsistent with the hypothesis that lime will function as a sticker by virtue of its *in situ* carbonation. The close correlation between the relative positions of the various supplements in the four columns of Table XV is an indication that the laboratory method provides an assessment of effect upon tenacity applicable to field conditions with a high degree of accuracy though, as was expected, the actual percentage figures have no quantitative significance.

TABLE XIV.

Initial Retention on Laboratory Surfaces.

Paraffin Wax.		Cellulose Nitrate.		Glyptal Resin.	
Supplement.	mg. spray per sq. inch.	Supplement.	mg. spray per sq. inch.	Supplement.	mg. spray per sq. inch.
Sulphite Lye	318	Petroleum Oil	186	Nil	204
Gelatine	246	Nil } Lime }	180	Lime	152
Petroleum Oil	218			Petroleum Oil	151
Nil } Lime }	200	Agral 2	87	Sulphite Lye (0·75%)	98
		Methyl cellulose	62	Methyl cellulose	84
Agral 2	185	Sulphonated Lorol	45	Agral 2	73
Sulphonated Lorol	157	Sulphite Lye	40	Gelatine	64
Methyl cellulose	131	Gelatine } Lime casein }	37	Sulphonated Lorol	60
Lime casein	75			Lime casein	58

TABLE XV.

Effect of Supplements upon Tenacity.

Laboratory Figure.				Field Figure.			
Cu₂I₂/cellulose nitrate.		Cu₂O/glyptal resin.		First application.		Second application.	
Lime casein	(93%)	Lime casein } Petroleum oil }	(101%)	Lime casein	(77%)	Petroleum oil	(91%)
Gelatine	(88%)	Gelatine	(100%)	Agral 2	(76%)	Agral 2	(85%)
Petroleum oil	(78%)	Nil	(92%)	Gelatine	(73%)	Lime casein	(78%)
Agral 2	(61%)	Agral 2	(91%)	Petroleum oil	(69%)	Methyl cellulose	(68%)
Methyl cellulose	(50%)	Lime } Sulphonated Lorol }	(87%)	Sulphonated Lorol	(66%)	Gelatine	(65%)
Nil	(28%)	Methyl cellulose	(86%)	Methyl cellulose	(61%)	Lime	(64%)
Lime	(23%)	Sulphite Lye	(65%)	Nil	(58%)	Sulphonated Lorol	(63%)
Sulphonated Lorol	(14%)			Lime	(54%)	Nil	(62%)
Sulphite Lye	(3%)			Sulphite Lye	(45%)	Sulphite Lye	(55%)
Cottonseed oil	(63%)			Cottonseed oil	(63%)	Cottonseed oil	(92%)

SUMMARY.

1.   The physical properties of spray deposits affecting the efficiency of protective insecticides and fungicides are discussed. The quantity of spray residue is determined by the initial amount deposited upon the sprayed surface (initial retention) and the ability of the deposit to withstand the action of the various agencies tending to its removal (tenacity). In addition, qualities of the spray deposit such as the uniformity of deposition (coverage) are concerned in relation to their effect upon the action of solubilizing agencies (availability) and upon tenacity.

2.   The initial retention of the solid particles of the suspensions examined is shown to be that of the aqueous phase, an indication that no preferential retention of the solid occurs.

3.   In the majority of the systems examined, the initial retention of the suspension is the same as that of the aqueous phase but, in a number of cases, the presence of solid particles reduces initial retention. This phenomenon appears to be related to the effect of the solid particles on the wetting and spreading properties of the spray, but it is shown not to be independent of the nature of the surface sprayed.

4.   The tenacity of the spray deposit is dependent upon the characters of the spray supplement, of the solid particle and of the surface sprayed. As a general rule, the degree of tenacity is determined by the relative ease of wetting of the spray deposit and, in accordance with this hypothesis, (i) tenacity is comparatively higher upon surfaces wetted with difficulty, (ii) supplements yielding residues insoluble in cold water, e.g. gelatine and lime casein, exert a favourable effect upon tenacity, whereas highly surface-active supplements, especially at high concentrations, exert a deleterious effect upon tenacity, (iii) oils, both hydrocarbon and glyceride, enhance tenacity.

5.   The laboratory method for the estimation of initial retention furnishes results in satisfactory agreement with field estimates based upon spray residue determinations.

6.   The laboratory estimates permit the arrangement of the spray supplements examined in order of favourable and unfavourable effect upon tenacity. This order is almost identical with that deduced from field trials.

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BACTERIOSIS OF CHERRY TREES :

RELATIVE SUSCEPTIBILITY OF VARIETIES AT EAST MALLING

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THE East Malling collection of cherry varieties was begun early in 1922 with trees of twenty-seven varieties kindly presented by Mr. E. A. Bunyard. Its primary object was the identification and classification of varieties, and particularly the study of varietal habit and behaviour.

The collection has grown rapidly ; at the end of 1936, though a few native and a number of overseas varieties had not yet been obtained, the names represented reached a total of 179 (155 sweet cherry, 12 " Dukes ", and 12 acid cherry). Of these a considerable number have not yet fruited ; it is already known, however, that at least twelve pairs of names are synonymous. On the other hand, at least eight names have to serve for two or more varieties each, no alternative names having yet been found. It is probable that the actual number of varieties in the collection is between 160 and 170.

The disease now known as Bacterial Canker early made its appearance. Within a few years it became evident that varieties differ widely in their susceptibility to the disease ; whilst a large number were moderately affected, some appeared to be almost immune, and others were so highly susceptible that it has hardly yet been possible to carry on a tree to the fruiting stage. A brief summary of the data so far collected, and a comparison with notes made elsewhere, may be of interest.

RELIABILITY OF OBSERVATIONS.

Whilst there can no longer be any shadow of doubt as to the existence of extreme differences in susceptibility between varieties, it is recognized that various factors may make the minor differences observed at East Malling less reliable as a guide to cherry planters. Even though, in the table of susceptibility given below, varieties planted since 1932 are omitted, it is obvious that, of the remainder, the older trees have run much more risk of infection than younger trees. Some trees or scions, also, may already have been infected when received, whilst others were healthy ; and it is plain that minor differences based on observation of not more than two or three trees of a variety cannot be completely trustworthy. When allowance is also made for the possibility, discussed below, that local conditions may influence the relative susceptibility of varieties, it will

not be surprising if the experience of others is in some cases widely different from that at East Malling.

INFLUENCE OF LOCAL CONDITIONS.

The soil of the four acre plot occupied by the older part of the East Malling variety collection varies considerably, particularly in its depth. This may in part account for the fact that the trees on a strip in the middle have been more severely affected by Bacterial Canker than have those elsewhere. But that this has not greatly affected relative susceptibility is shown in two ways. In the first place, the two trees of White Heart, which have so far proved highly resistant, are in this strip, and have long been surrounded by badly diseased trees. Secondly, in a number of cases, the original tree of a variety was planted on a headland, far from this strip, and the two trees " worked " from it were planted on the strip. In no such case was the apparent susceptibility of the variety much altered.

It is indeed interesting to observe how consistently the two trees of each variety " worked " at East Malling have reacted similarly to Bacterial Canker. As a rule, the two trees have been planted close together ; but where one has died, another has sometimes been planted at a distance. The instances of severe damage to one tree and little to the other are very few, and have nearly all occurred whilst the trees were young ; as time elapses, the reaction of the two trees invariably tends to be more similar. This has occurred even where varieties planted under two names at a considerable distance apart have eventually proved to be identical. In at least one such case, referred to below, a peculiar type of injury from Bacterial Canker led, indeed, to the identification of a variety.

Although the present writer's experience of cherry Bacterial Canker in conditions different from those at East Malling is rather limited, he has observed several instances where groups of varieties have shown a relative susceptibility closely similar to that at East Malling. The exceptions are few, and perhaps merit a short discussion.

The first concerns Géante d'Hedelfingen. This variety, received at East Malling under this name and also as " Bradbourne Black ", has proved extremely susceptible to Bacterial Canker. It has never yet been possible to obtain a head more than three years old from the crotch ; consequently there has been little fruit, but enough, fortunately, to establish the correctness and synonymity of the names. Not more than two miles from East Malling is an orchard containing Hedelfingen, Early Rivers, Kent Bigarreau and Napoleon Bigarreau, the trees being perhaps 25 or 30 years old. Of the four varieties, Napoleon seems likely soon to be wiped out by Bacterial Canker ; Kent Bigarreau is considerably injured, Early Rivers very little, and Hedelfingen not at all.

Since the fruit of Hedelfingen is easily identified—there being, so far as the writer knows, no closely similar variety—it seems that in this case local conditions must have greatly modified its susceptibility. The other three varieties have behaved almost exactly as East Malling experience would have led one to expect. It is possible that the chief factor in the resistance of Hedelfingen is age of tree. It has frequently been stated that if trees can be carried on beyond a certain age they become resistant to Bacterial Canker, or at least to " gumming ". Whilst none of the trees at East Malling is old enough to provide evidence on this point, it seems from the experience of growers that it is seldom if ever true of Napoleon Bigarreau and some other varieties. But it may be true of Hedelfingen. The only alternative explanation would appear to be that local conditions—presumably soil or cultivation—can radically alter the relative susceptibility of varieties. Cherry growers in Kent have long believed that trees in grass, or, in fact, under any conditions that tend to reduce vigour of growth, are less susceptible to " gumming " than trees that are induced by cultivation or other means to grow rapidly. The opportunity to test this theory has not so far occurred at East Malling. It is possible that it may be more applicable to some varieties than to others.

A second case occurred on an isolated fruit farm in the south of England. A large area planted with standard sweet cherries had, within four or five years of planting, been very severely injured. The soil was somewhat variable, particularly in its depth ; but there did not seem to be any considerable areas where the trees were less severely affected.

Half a mile from the edge of this orchard, to the west, was a small area of bush cherries, of many varieties, including, it was understood, at least three of the varieties in the orchard. The trees came from a different source, and were planted a year later than the large orchard. The soil appeared similar, and equally variable. In the whole of this plantation not a single instance of infection with Bacterial Canker could be found.

For such a striking difference, perhaps, no single explanation could be adequate ; a variety of circumstances may have contributed to it. But since the trees in the large orchard were already dying in large numbers within four years of planting, and since no local sources of infection could be found— although the neighbouring woods contained many fine healthy wild cherries—it seems possible that the initial infection came with the trees, and that those in the small plantation were healthy when planted. Perhaps the fact that the small plantation was to the west of the large orchard, the prevailing winds being west and south-west, had saved it from infection up to the time of the writer's visit. There is some slight evidence which suggests that bacterial infection can travel considerable distances on the wind, even where no cherries or other host plants intervene.

A third case, referred to by Dr. Wormald on a subsequent page (p. 37), concerns Roundel. As the explanation here is likely to be concerned with incorrect nomenclature, the case is discussed under another heading below.

DEGREE OF SUSCEPTIBILITY AT EAST MALLING.

The following classification of varieties according to their susceptibility to Bacterial Canker will at least serve as a rough guide. It is based entirely on field observations ; very few have been confirmed by laboratory examination for the presence of bacteria. The symptoms of Bacterial Canker, however, are usually distinct enough to ensure at least a low proportion of error in observation. Since gumming in cherries, and even the death of branches or whole trees may be due to more than one cause—e.g. infection by Stereum or Verticillium—there may have been a very few mistakes. But it is most unlikely that mistaken observation could account for any but a few of the minor differences shown in the Table.

In the Table the groups are given in order of decreasing susceptibility. Group 1, " extreme ", includes only varieties of which more than one tree has either been completely killed, or has had to be cut down below the crotch. Most of these varieties have been cut down or replanted more than once. One variety, referred to below, has been completely lost ; several have been saved only by constant reworking in the nursery.

The varieties in Group 2, " considerable ", have in all cases had one tree either killed or cut down below the crotch. In nearly every case the other tree or trees have been considerably but less severely injured.

The third group, " slight or moderate ", includes those varieties which have at times lost a branch of at least considerable size, but have neither had to be repeatedly cut nor have lost whole trees.

In Group 4, " very slight ", are placed those varieties which have never yet had a branch infection of any consequence. A few, as for instance, Newington Black, have at times had a considerable number of spurs infected and killed ; others have sometimes been moderately or even severely affected by Bacterial Leaf Spot. But experience has shown that, as far as branch, crotch, or stem infections are concerned, none of these varieties has been seriously injured.

It is quite certain that longer experience will make it necessary to transfer some of the less susceptible varieties to a more susceptible category. At the end of 1935, for instance, Black Tartarian E and Waterloo would have been included in Group 4 ; in 1936, however, all three trees of Black Tartarian E, and one tree out of three of Waterloo lost branches of good size, clearly through Bacterial Canker ; they were therefore transferred to Group 3. It may eventually be necessary to transfer some varieties to a less susceptible group ; only long experience can show the need for this.

Table of Susceptibility.

1. Extreme.	2. Considerable.	3. Slight or moderate.	4. Very slight.
	SWEET CHERRIES.		
Abundance.	Black Downton.	Baumann's May A.	Bauman's May B.
Bedford Prolific B.	Burr's Seedling.	Bedford Prolific A.	Big. de Jaboulay
(not Roundel).	" Early Purple Gean."	(? = Roundel).	(= Early Lyons and
Beeve's Heart.	Goodnestone Black.	Belle Agathe.	Frühe Rote).
Belle de Droures.	Great Bigarreau.	Black Eagle.	Black Cluster.
Big. de Mezel.	Guigne très Précoce.	Black Elton.	Black Oliver.
Big. de Schrecken.	Hooker's Black.	Black Tartarian E.	" Buttner's Rote "
Bing.	Hoskin.	California Advance.	(White).
Bloor's Heart.	Kent Bigarreau	Circassian.	Caroon A.
Burbank.	(= Amber Heart).	Cryall's Seedling.	Cleveland Bigarreau.
Centennial.	Knight's Bigarreau.	Dunn " Mazzard ".	Early Cluster.
Chapman.	Ludwig's Bigarreau.	Early Amber.	Emperor Francis
Early Bigarreau.	Norwegian.	Early May	(1 tree).
Florence.	Ord.	(= Mumford).	Frogmore Bigarreau.
G. d'Hedelfingen	Pontiac	Early Rivers	Ham Green Black.
(= Bradbourne	Ramon Oliva.	Elton Heart.	Hollander.
Black).	Royal Queen.	Gov. Wood.	Kassin's Frühe.
" Grosse Schwarze	St. Margaret's	Knight's Early Black.	Lambert.
Knorpelkirsche."	(= Noble).	Lulsley Early	Large Black
Guigne d'Annonay.	" Thamenkirsche ganz	Black (? = Früh-	" Mazzard ".
Ironsides	Süsse Schwarze "	este der Mark).	" Longley's Black
(= " Ohio	Wellington A.	" Noir de Schmidt "	Eagle."
Beauty ").		(White).	Maiden's Blush.
Late Amber.		Old Black Heart A.	Newington Black
Lewelling.		Old Black Heart B.	(= Caroon B).
West Midlands		Philpott's Favourite	Noir de Guben.
Bigarreau.		(= Big Reverchon).	Norbury's Early
Yates' Seedling.		Rockport Bigarreau.	Black.
Yellow Spanish.		" Schneider's Späte	Nutberry Black.
		Knorpelkirsche "	Perserving
		(early).	" Mazzard ".
		Smoky Dunn.	" Red Turk."
		Strawberry Amber.	Rodmersham
		Turkey Heart.	Seedling.
		Victoria Black.	Roundel.
		Wellington B.	Small Black
		Windsor.	" Mazzard ".
			Sutton's Purple.
			White Heart.
	" DUKES."		
Planchoury.	" Belle de	Archduke.	" Belle de
	Franconville."	Ronald's Late Duke.	Chatenay."
	May Duke.	" Rote Mai "	Belle de Choisy.
	Reine Hortense.	(? = Ronald's Late	Empress Eugenie.
		Duke).	Nouvelle Royale.
			Olivet.
			Royal Duke.
	ACID CHERRIES.		
None.	Flemish Red.	Morello A.	Carnation.
	Griotte de Portugal.		Coe's Carnation.
	Gros Gobet		Kentish Red
	(= Montmorency		(Self-fertile).
	Short Stalk).		Kentish Red
	Wye Morello.		(Self-sterile).
			Morello B (and
			others ?).
			Ostheimer Weichsel.
			Triaux.

Several varieties added to the collection more recently than those in the Table have already proved distinctly susceptible to the disease. These include August Heart, Beechcroft, Bowyer Heart, Dangler, Leicester, Napoleon Bigarreau, Peggy Rivers, Pointed Heart, Ronald's Heart, Sutton's Prolific (distinct from Sutton's Purple), Werder's Early Black, and two late White Cherries not yet identified. It is not unlikely that some of these will eventually prove to be less susceptible than they now seem.

QUESTIONS OF NOMENCLATURE.

Nearly all the names given in the Table are believed to be correct. Where two or more varieties are entered under one name—no alternative name for either having yet been found—they are distinguished by letters. Black Tartarian E is the variety distinguished in this way by Crane (1). No other '' Black Tartarian '' has yet been in the East Malling collection long enough to be included.

In a few cases the names given are clearly wrong ; these are placed in quotation marks. The variety received as '' Buttner's Rote '' (properly a black : cherry), is white, as is also the variety received as '' Noir de Schmidt ''. Until these varieties can be identified, it seems best to continue using the incorrect names.

The synonyms given (except those queried) have been confirmed.

A case of apparent difference in susceptibility of a variety under different conditions, mentioned by Dr. Wormald on a subsequent page (p. 37) of this Number, probably arose through incorrect nomenclature. '' Roundel '' was there included amongst the more susceptible varieties. At East Malling, and in the experience of several growers, Roundel is found to be one of the most resistant. In all probability, two different varieties are here concerned. There is a group of varieties, including apparently at least two called '' Bedford Prolific '' (or '' Bedford Black ''), which are closely similar to Roundel in every way. In 1923 East Malling received buds of '' Roundel '' and '' Bedford Prolific '' which were supposed to be identical ; and in 1924, from another source, a tree of '' Bedford Prolific ''. Of these, '' Roundel '' has grown fairly well, has always been healthy, and has been bearing increasing crops for some years. In tree and fruit characters it seems identical with the Roundel commonly grown. The first '' Bedford Prolific '' ('' B '') proved so highly susceptible to Bacterial Canker that both trees were killed in spite of efforts to save them, and the variety was lost to the collection. The second '' Bedford Prolific '' ('' A '') is in many ways very similar to Roundel, but appears to differ in producing a slightly more spreading tree ; its blossoming season is consistently somewhat earlier, and it appears rather less resistant to Bacterial Canker than Roundel. Except for these differences, it could so far easily be mistaken for Roundel.

The susceptible " Roundel " mentioned by Dr. Wormald may therefore have been one of these " Bedford Prolifics ".

VARIOUS TYPES OF BACTERIAL INFECTION OF CHERRIES.

The attempt to control Bacterial Canker by cutting out infections has yielded a mass of observations on the susceptibility of varieties to various types of infection.

From the grower's or practical point of view, the types of bacterial infection most commonly met with are : Leaf spot, bud infections on shoots of the previous summer (presumably through the petiole scar), spur infections, together with infections of the bark and cambium of branch, crotch and stem, leading usually to cankers. Varieties differ not only in their susceptibility to infection in general, but also in the relative degree to which they are subject to these various types of infection. It has been observed for several years, for instance, that of two varieties in the East Malling cherry rootstock trials, Early Rivers is rather highly susceptible to leaf spot and little to canker, whilst Bigarreau de Schrecken is comparatively resistant to leaf spot but susceptible to canker. The first of these observations confirms that recorded by Dr. Wormald on a subsequent page (p. 41).

Whilst this observation on Early Rivers has frequently been confirmed, certain other observations of peculiar types of infection occurring chiefly on one or two varieties are more occasional, and are not always confirmed by subsequent experience. Thus, in the spring of 1930, it was observed that the two trees of " Bedford Prolific B " had most of their flowering spurs killed, apparently by bacteria, but were otherwise little injured. This observation was not repeated ; in 1932 both trees developed stem or crotch infections, which spread so rapidly that, in spite of repeated cutting back, both trees died in 1933, without providing scions for further propagation.

In the spring of 1931 a peculiar type of spur infection not previously seen was noticed on the two trees of the variety known in Kent (probably incorrectly) as " Ohio Beauty ". On many spurs certain flower buds failed to grow, whilst other buds produced only small and poorly formed flowers ; most of these spurs were completely killed before any fruit developed. Shortly afterwards the same type of infection was found on the two trees of the Herefordshire variety " Ironsides " ; it was not found in that year on any other variety. This was the first clue to the identity of these two varieties, subsequently confirmed by fruit and stone descriptions.

In the summer of 1935 several varieties developed many spur infections, but were otherwise almost uninjured, except in some cases by leaf spot. Of these the most interesting was " Newington Black ", a name usually given as a synonym for Caroon. Both of the trees of this variety had many spurs killed,

whilst those of the variety received as "Caroon A", close by, were both practically uninjured. Careful observation of the fruit in 1936 made it seem very probable that the two varieties, though closely similar, are not identical. It may be remarked here that both these varieties, received from the Sitting-bourne district, are much later in ripening that the "Caroon C" found in old orchards near East Malling. Though Hogg (2) and Bunyard (3) disagree in certain particulars, they agree in describing Corone (Caroon is regarded as a local name) as late ripening.

A type of infection observed in certain seasons in low-worked nursery trees of Early Rivers (rarely in older trees and those of other varieties), is, in the writer's experience, unique amongst fruit tree diseases caused by micro-organisms. It seems quite clear that considerable areas of bark on the tree stem may be invaded by the bacteria, even though the cambium is practically untouched. Such infections do not appreciably injure the tree, but they make it, in bad cases, so unsightly that it could not be sold. A moderate proportion of the Early Rivers trees raised for the East Malling rootstock trials had this type of infection in 1927. Some of the less unsightly specimens were planted in the trial ; they soon developed smooth stems showing no sign of the injury. This type of infection has not so far been specially examined for the presence of bacteria ; but the writer is in no doubt that the cause is the same as that of the very large number of bacterial infections he has examined.

Still another type of infection has been found, chiefly in Morello. The variety grown as Morello ("A") in the rootstock trials came from a different source, and appears in several respects slightly different from the Morello ("B") in the variety collection. Of the former, two or three trees out of more than two hundred have been lost, apparently through Bacterial Canker ; but the variety is generally resistant, except to one type of infection. In cutting out infections of Brown Rot, the writer has frequently found dead or dying twigs in which Brown Rot had clearly nothing to do with the injury. In practically every case there was a small infection, girdling the twig, at the tip of one year's growth and the base of the next. Such infections, mostly found in May and June, were usually at the base of the shoot formed two years previously. The twigs at the point of infection were rarely more than 6 to 8 mm. in diameter, and often much less ; the injury to the tree was therefore slight.

ROOTSTOCK INFLUENCE ON SUSCEPTIBILITY.

In view of the fact (4) that certain apple rootstocks have appeared to influence the susceptibility of the scions worked on them to various fungus diseases, it is at least possible that cherry rootstocks might influence the susceptibility of the scion to Bacterial Canker.

Observation, however, strongly suggests that any influence there may be is not direct, due in some way to the union of resistant with susceptible tissues, but indirect. If it can be shown to exist, it will probably be associated with an influence of the rootstock on the vigour of the scion, presumably through some nutritional factor.

Since nearly all the sweet cherries so far planted at East Malling have been "low-worked", with stems of the scion variety, the union occurs practically at ground level, and does not provide a good opportunity for observing the possible extension of a bacterial infection into the rootstock. The large young orchard already mentioned as having been very severely injured by Bacterial Canker, however, consisted entirely of "high-worked" trees, the stem being formed by the stock. Here some interesting observations were made on the relative susceptibility of stock and scion.

Whilst some of the stocks were much damaged by the disease, several were clearly very resistant, and in these cases the scion was as clearly highly susceptible. The bacteria had invaded apparently every cell of the scion tissue, but seemed to have been completely unable to invade the stock tissue. The resistant stock tissue did not appear to have made even the small amount of scion tissue in direct union with it any more resistant than it normally would have been. There does not therefore seem to be much hope of increasing the resistance of susceptible varieties by either low or high working on the stems of resistant stocks. These stocks may, however, be useful for "frame-working" susceptible varieties; they could not be expected to prevent the death of certain branches down to the point of union, but they should prevent crotch infection, and the subsequent loss of the whole tree.

SUMMARY.

Observations on the East Malling collection of cherry varieties have shown that:—

1. Varieties differ widely in their susceptibility to Bacterial Canker.

2. There are several types of infection, of leaf, spur, branch and stem, and varieties differ in their relative susceptibility to these various types.

3. Whilst rootstocks may indirectly influence the susceptibility of the scion, they do not appear to influence it directly; susceptible scions on resistant rootstocks may be completely killed to the point of union, though the stock is entirely uninjured.

4. The reliability of the observations, and certain questions of nomenclature, are discussed, as is also the possibility that local conditions may influence the relative susceptibility of varieties.

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BACTERIOSIS OF STONE FRUIT TREES IN BRITAIN

VI. FIELD OBSERVATIONS ON BACTERIOSIS OF SWEET CHERRY TREES

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GENERAL OBSERVATIONS.

For many years there have been serious losses in cherry orchards in the south-eastern and western fruit-growing counties of England, young trees dying completely and old established trees losing large limbs. The cause of this so-called " die-back " was long disputed, but during recent years evidence has accumulated which shows that much of it is a result of bacterial infection, and, in fact, that is has much in common with a similar disease of plum trees in which one form of die-back is now known as Bacterial Canker because it has been found to be due to bacterial lesions on branches and stems (18, 19). Similar bacterial lesions are associated with die-back in cherry trees although in cherries the exudation of gum from such lesions is usually far more profuse than in plum trees. Gummosis itself has been attributed to various unfavourable factors ; it would appear that any condition which arrests the downward flow of elaborated food material will induce gummosis in cherry trees, and one of the most potent of these factors is bacterial infection which produces cankers on branches and stems.

The association of bacteria with die-back in cherries in Britain first came to the writer's notice as long ago as 1919 when his attention was drawn to the condition of a cherry orchard at Ham Street, near Ashford, Kent. On visiting the orchard, in June of that year, trees of Black Tartarian were seen with many dead branches, some of which had been dead for some time while others were just beginning to show evidence of disease by yellowness of foliage and wilting of leaves and fruit. The bark of the affected branches, when cut, was seen to be quite brown, and a microscopic examination of the cortical tissues towards the lower extremities of the lesions showed dense bacterial masses. Further back the xylem also was discoloured and fungal hyphae were present in the vessels. As bacteria only were found in tissues bordering on the healthy parts it seemed likely that they were the primary cause of the damage and that the fungus in the xylem was secondary.

In May 1923, a similar condition was seen on a fruit farm in East Sussex. In this case die-back was accompanied by severe spotting of the leaves, and

the spots when teased out in water yielded bacterial masses, thus suggesting that there might be some connection between the leaf spotting and die-back. One orchard consisted of four varieties, Cluster Black Heart, Early Rivers, Waterloo and Black Tartarian, all affected to a greater or less extent. The Cluster Black Heart was worst, only a few trees appearing quite healthy ; many had branches killed back almost to the crotch and some trees had been headed back, because of the disease, and regrafted. At least half the trees of this variety were seriously affected and had one or more dead branches, while others showed leaf spots only. The typical appearance was for a branch to have a dead terminal portion (one-year-old) bare of leaves ; lower down the young shoots bore some leaves which were spotted and others completely blackened. The spots eventually fell out leaving " shot-holes ". There was evidence that some of the flowers had been attacked, for they were withering, their pedicels being wholly or partly brown and containing bacteria. Certain Early Rivers trees were also seriously affected but, on the whole, this variety suffered less than Cluster Black Heart. On Waterloo and Black Tartarian the disease was less severe.

In another orchard on the same farm a few young trees of Black Eagle were affected. The spots on the leaves of this variety were reddish brown, circular, usually with a lighter translucent centre, and up to about 1·5 mm. in diameter.

On Black Tartarian leaves the spots were generally larger (up to 3 mm. in diameter) and more scattered ; " shot-holes " were very numerous in some leaves.

Another visit to the Ham Street orchard, in 1923, showed that here, too, the dying back of the branches of the Black Tartarian trees was accompanied by bacterial leaf-spotting, thus offering further evidence of the connection between the two symptoms. The owner said that, until that year, there had been little die-back since the outbreak in 1919. The abnormally cold damp weather of 1923 was probably responsible for the severe leaf infection in that year. Spotted leaves, often with withered margins, were common. Other varieties in the same orchard were Early Rivers, Napoleon and Turk, and these were apparently free from the disease, though observations in other localities showed that these varieties also are susceptible.

In the same year (1923) bacterial leaf spot of cherries was seen in several localities, again usually associated with die-back. In an orchard of three-year-old trees at Bramling, near Canterbury, it was accompanied by serious dying back of the branches, many trees having a " stag's head " appearance because of the dead leafless branches ; others were dead down to the crotch and in such cases the whole scion head of a tree was killed, the stock-stem only being alive. One tree had a lesion two feet long on the " Gaskin " stock-stem ; it had yellowish foliage and was evidently dying. Fungal fructifications of a

species of *Cytospora* were found on the discoloured bark ; towards the extremities of the lesion, however, no mycelium could be found, but numerous bacterial rods in dense masses were present. Most of the trees showed severe leaf spotting and on some of them, the spots were not only on the foliage of the scion head but also on the leaves of the " feathers " on the Gaskin stem.

This orchard was visited during the following year and the disease again found to be rife. It was ascertained that the orchard consisted originally of three hundred and twenty trees, planted on the site of an old hop garden in the winter of 1920-21. In 1922 about twenty had shown the disease, so these had been grubbed and replaced by others, but in 1923 it was found necessary to grub forty more. Most of the trees remaining in 1924 showed at least a trace of the disease. A few with small yellowish leaves were dying and had stem lesions, but most of the trees showed a dying back of the branches, the terminal portion of one or more branches being dead and bare of leaves, while the lower parts, below the lesions, bore spotted, but otherwise normal, leaves. Of the varieties grown, Roundel Heart* was the first to succumb and the gaps had been planted up with Bigarreau de Schrecken which also became attacked. The disease was severe on Black Eagle, less pronounced on Waterloo, whilst Early Rivers, Turk and Amber Heart were only slightly affected. Again it was found that although fungal hyphae were present in the diseased parts of stems and branches, the tissues bordering on the healthy parts contained bacteria only which oozed out in dense masses on teasing out portions of the cortex in water.

More recent observations have been made on the variety trial plots of cherries at the East Malling Research Station where certain varieties have proved to be very susceptible to bacterial infection.† All parts of the trees except the roots may be attacked, for lesions have been found on stems, branches, shoots, buds, leaves, flowers and fruit. These different forms of damage have come under examination and organisms associated with the various types of lesions have been isolated.

SYMPTOMS OF BACTERIOSIS IN SWEET CHERRY TREES IN BRITAIN.

Stem and Branch Lesions.

As in plum trees, the bacterial infection on stems and branches of cherry trees induces necrosis of the cortical and phloem cells, accompanied by

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* In this plantation the variety known as Roundel Heart was apparently very susceptible. In an orchard at Wye (see p. 41) Roundel Heart showed less infection than most of the other varieties, and this relatively low susceptibility has been observed at East Malling also. In the nomenclature of cherries there are many anomalies and it is possible that the variety at Bramling was not the same as that grown at Wye under the same name.

† The field observations at East Malling have been carried out in collaboration with Mr. N. H. Grubb whose account (8) of the relative susceptibility of the varieties examined will be found on another page (p. 25).

gummosis. In cherries the gum is usually very copious, and in wet weather it oozes out to form translucent masses on stem, crotch or branches. The cambium is destroyed so that, when a lesion does not completely girdle the stem or branch, development is arrested over the affected area and the continued growth of the parts not attacked results in the infected region remaining sunken below the general level. As a rule a lesion does not increase in size after June, and if a branch is not girdled by that time the unattacked tissues round the margin of the lesion develop callus which tends to cover up the cankered area. Such cankers may cause little or no permanent injury.

During July and August, therefore, there is usually a border of callus to such a lesion, the bark cracks and a canker results. In these cankers the organism dies out, and attempts at isolation during July and August may fail. It has been observed, however, that some lesions do not produce callus so early and the organism has been isolated from cankers in late July and early August. Such lesions were observed during the rather cold and wet summer of 1936, and it would seem that these conditions prolong the development of the bacteria in cankers during summer.

A girdling canker, destroying the cortex, phloem and cambium, but not at the same time the xylem, may not check the ascent of sap while arresting the downward flow ; the branch thus becomes " ringed " and the accumulation of foodstuffs immediately above the lesion induces increased development of the tissues in that region so that just above the canker the branch is abnormally thickened (Fig. 6). Eventually, however, on the parts terminal to a girdling canker, the foliage turns pale green or yellowish, tends to be " curled ", and finally withers. The final result therefore of a girdling canker is to kill those portions above it.

On a maiden tree the infected region on the stem is indicated by failure of the buds to develop there. Above the lesion the tree may come into leaf but the leaves turn yellowish, are narrow and curled, and eventually wither (Figs. 4 and 5). Below the canker the buds are stimulated to increased development and they grow out to form strong healthy shoots, often, however, with spotted leaves as a result of infection by bacteria splashed from the canker.

Bud and Spur Infection.

Isolated buds are often killed and one-year-old twigs may be seen with one or more buds failing to develop while the rest of them grow out normally and come into leaf. In common with other lesions on infected trees these dead buds contain numerous bacteria. There is usually a lesion round the base of a dead bud and if this is extensive it may girdle the twig. The dying back of one-year-twigs as a result of these nodal infections is a common

occurrence, particularly on young trees, and on maiden trees the "leaders" may be killed back in consequence.

The buds on the spurs of older branches may also become infected. One or two buds only on a spur may be killed but often the axis of the spur is invaded and all the buds are destroyed. Again, such a lesion may extend into the branch itself, producing a canker which may girdle and kill the branch. Similarly on two-year-old trees, the main stems become infected from the spurs.

Some varieties appear to be more susceptible to spur infection than any other form of bacterial attack. In 1935 at East Malling several varieties had many spurs killed but no other serious damage ; on some of the trees even leaf spot infection was slight.

Bacterial Shoot Wilt.

Blackened lesions sometimes appear on the young green shoots. As they are usually much longer than broad they may extend for several centimetres along one side without actually girdling. Such a lesion causes a distortion of the infected shoot which tends to curve over towards the side bearing the lesion, but frequently shoots are girdled and the terminal leaves wither. Bacterial shoot wilt is almost invariably accompanied by severe leaf spotting.

Similar lesions have also been observed on shoots in nursery stool beds, but bacterial shoot wilt in stools and layer rows appears to be rare, although wilting due to other causes occurs.

Leaf Infections.

Bacterial infection of the foliage usually takes the form of leaf spots. When these are numerous and crowded they are very small and angular ; when more scattered they are larger, 1·5 to 3 mm. in diameter, and circular. The spots at first have a water-soaked appearance, but later the dead tissues turn brown and drop out leaving " shot-holes ". If the tissues of a young spot are teased out in water, bacteria ooze out in a dense gelatinous mass which may be seen under fairly low power of the microscope (2/3" objective, and eyepiece ×6), although higher magnification is necessary to show the bacterial rods. If young spots are examined in very wet weather some of the bacteria may be seen to be actively motile ; usually, however, the only movements they show are the passive oozing out from the ruptured cells and " Brownian " motion.

When the spots are numerous they sometimes coalesce to form irregular necrotic blotches causing the leaves to become distorted. Often the spots are crowded towards the drooping tips of the leaves, suggesting that the accumulation of rain water at the tips brings the bacteria to this part of the leaf and encourages infection. What appears to be infection through the marginal

"water pores" (hydathodes) has been observed, and the withering of the leaf margins sometimes seen appears to be an extension of such infection from the "teeth" into the adjoining tissues.

Infection of petioles and midribs also occasionally occurs. This is a result of the extension of the bacteriosis from an infected spur upwards along the petioles into the laminae; from the midrib it may then extend into the larger veins. Infection of the leaves in this way is unusual, however, and has been seen only in very wet weather in early spring before the leaves have fully expanded. When there is much infection about the time when the buds are expanding or soon after, the young leaves may be killed before they fully expand.

Leaf infection is usually severe in the neighbourhood of branch lesions, particularly on leaves borne on spurs immediately below the cankers; this suggests that the bacteria are carried down by rain from the cankers on to the foliage beneath.

Infection of Flowers.

Occasionally wilted flowers are found with bacteria within the tissues. Whether flowers may become infected directly from without, or whether they are only invaded from the spur on which they are borne has not been ascertained. This condition is rarely seen on sweet cherries.

On acid cherries there is a more serious blossom blight which, from the presence of numerous bacteria within the discoloured organs, appears to be a bacterial disease; the organism usually isolated from these lesions is different, however, from both of those causing bacteriosis of sweet cherries in Britain, but its identity has not yet been determined.

Fruit Infection.

Lesions containing bacteria have been seen on the young green fruit, but this condition, too, is rare. It has been found on cherries in three localities but only on a few fruits, so that there is no reason to suppose that this form of infection is likely to cause any serious loss.

Varietal Susceptibility.

Observations in commercial plantations, as recorded above, show that certain varieties of cherry commonly cultivated in Britain are very susceptible to bacteriosis. Among the most susceptible would appear to be Black Tartarian, Cluster Black Heart, Bigarreau de Schrecken, Black Eagle and (as shown below) Bradbourne Black, while Waterloo, Turk, Amber Heart and Early Rivers are also sometimes seriously attacked.

Detailed records taken in an orchard at Wye, near Ashford, Kent, during the summer of 1936, provided information on the relative susceptibility of

certain cherry varieties to bacterial infection. Over three hundred and fifty trees, including nine varieties,* were examined individually and the degree and type of infection noted. The number of trees cankered in each variety was recorded, and the degree of leaf spotting was determined by using the numbers 0 to 4, 0 indicating no infection, and 4 very severe spotting involving most of the leaves.

The varieties, and their order in increasing degree of infection with regard to cankering (stem and branches) and leaf spot, are here shown.

Cankering.	*Leaf Spotting.*
Frogmore.	Frogmore.
Governor Wood.	Governor Wood.
Roundel Heart.	Roundel Heart.
Early Rivers.	Amber Bigarreau.
Napoleon.	Emperor Francis.
Waterloo.	Napoleon.
Amber Bigarreau.	Waterloo.
Bradbourne Black.	Bradbourne Black.
Emperor Francis.	Early Rivers.

Frogmore proved to be the most resistant to both cankering and leaf spotting. On the thirty-four trees of this variety there was only one tree with canker—a small stem lesion causing no appreciable damage—and the trees had very little leaf spot, thirty of them showing no leaf infection, and the other four only a trace.

At the other end of the list is Emperor Francis of which seven trees were cankered out of twenty-seven or 26 per cent. ; Bradbourne Black proved also very susceptible, with very severe leaf spotting and 25 per cent. of the trees cankered. Leaf spotting was particularly bad on Early Rivers, a variety which is evidently very susceptible to this form of infection, although cankering was not so severe as on most of the other varieties in this orchard (three trees cankered out of forty-three).†

On the whole it will be seen that if we except Early Rivers there is a fairly close relationship between susceptibility to leaf spot and degree of damage by cankers. This is particularly noticeable in the varieties Frogmore and Governor Wood, which appear at the head of both columns as showing little infection, and, at the other extreme, in Bradbourne Black which is very susceptible to both forms of infection.

* The writer is greatly indebted to Mr. W. G. Kent and Mr. C. R. Thompson for their help in taking these records.

† At East Malling also, Early Rivers has proved to be very susceptible to leaf spotting. Of three varieties (Early Rivers, Turk, and Bigarreau de Schrecken) grown on one plot here, Rivers is the least affected by canker and most by leaf spot. Rivers is generally regarded by growers as relatively resistant to the canker form of infection.

With regard to the shoot wilt form of bacteriosis the greatest number of affected shoots were found on Napoleon and Waterloo, fewer on Amber Bigarreau, Bradbourne Black and Roundel Heart.

ORGANISMS CAUSING BACTERIOSIS OF CHERRIES IN BRITAIN.

Many isolations have been made from the stems, branches, buds and leaf spots, of infected trees at East Malling and in other localities, and inoculation experiments on stems, branches and leaves with these organisms have shown them to be pathogenic. An account of these experiments will be published later, but it may be stated here that the bacteria isolated fall into two groups, one conforming to *Pseudomonas prunicola*, which has been shown to be the cause of a shoot wilt of plum trees at East Malling, the other to *P. mors-prunorum* which produces Bacterial Canker in plum trees. Each of these organisms has been isolated from stem and branch lesions and from leaf spots on sweet cherries.

P. prunicola has been obtained several times from lesions on trees at the East Malling Research Station, once also from a branch lesion on a commercial fruit farm at East Malling and once from spots on fruit obtained from North Kent, while *P. mors-prunorum* has been isolated many times from specimens collected at East Malling and a number of other localities. It would appear then that *P. mors-prunorum* is the more generally distributed of the two organisms in Britain. On the other hand *P. prunicola*, or a form which appears to be indistinguishable from it, occurs in America ; moreover it is closely related to and perhaps identical with the lilac blight organism *P. Syringae*, which has been found on the Continent, in North America and also in this country. Reference to the work by American investigators who are studying the relationship of these organisms is given below.

On acid cherry trees at East Malling bacteria have been found associated with lesions on branches, with leaf spots, and in withered flowering spurs. The organisms isolated from these lesions are under investigation. One of them, from branch cankers, appears to be identical with *P. mors-prunorum*, but a detailed comparison has not yet been made ; another, isolated from leaf spots and blighted flowering spurs, is different from both *P. prunicola* and *P. mors-prunorum*, as well as from *P. Pruni*, found by Dunegan to cause leaf spotting of Morello cherries in America, but its identity has not yet been determined.

BACTERIOSIS OF CHERRY TREES IN OTHER COUNTRIES.

In 1902 Brzezinski (4) found bacteria associated with cankering and gummosis in stone fruit trees in Poland, and concluded that in peaches, plums and apricots gummosis was caused by an orange yellow organism while in cherries it was an organism of which he stated, " ses colonies, obtenues en culture, sont blanchêtres, fortement opalescentes et particulières par leur structure ", but he offered no further description of the organisms.

In Germany, in 1905, there was an epidemic outbreak of gummosis and high mortality in cherry trees. The disease was investigated by Aderhold and Ruhland (1, 2) who isolated a bacterium from the lesions and succeeded in reproducing the disease by inoculations with this organism, which they named *Bacillus spongiosus*. The symptoms of the disease as affecting stems and branches agree with those observed in this country, but there is no mention of leaf infection ; hence the leaf spot phase if present was either not observed or, if noticed, was not considered to have any connection with the stem and branch lesions.

In the western States of North America bacterial gummosis is a serious disease in cherries, and it has come under the notice of several investigators. Griffin (7) was the first to show that this condition in Oregon was caused by bacteria ; he wrote, " It is in the more humid portion of western Oregon that its prevalence and destructiveness gives it the rank of a major disease." He isolated and described the organism, which he named *Pseudomonas cerasus*, and by inoculation experiments proved it to be the cause of the disease.

These results were confirmed by Barss (3) who gave a detailed description of the disease. His account of the cankers, the " blighting of buds and spurs ", and leaf infection, shows that the symptoms he observed are similar to those found in Britain.

The bacteria causing gummosis and sour-sap of stone fruit trees in California have been closely studied by E. E. Wilson (13, 14) who finds that there are two nearly related organisms which may cause these diseases. One is probably that described by Griffin, *Pseudomonas Cerasi*,* an organism which produces a green coloration in beef extract media, and a white organism which Wilson finds is similar to *P. prunicola*, described by the present writer as causing a wilt of plum shoots in Britain (17). By reason of the close affinity of the two organisms Wilson prefers to consider *P. prunicola* as a variety of *P. Cerasi* and names it *P. Cerasi* var. *prunicola*.

Other organisms have been found to cause bacteriosis of cherries in North America. Sackett (11) described a disease of the Wragg Cherry, and the bacterium causing it he named *P. Cerasi Wraggi*. More recently Dunegan (6) has described a leaf spot of Morello cherries which he finds is caused by *Bacterium (Pseudomonas) Pruni*, an organism which had previously been shown to cause serious leaf spotting of plums and peaches in North America.

C. O. Smith (12) has called attention to the close relationship not only between *P. prunicola* and *P. Cerasi* but also between these and *P. Syringae* (the cause of bacterial blight in lilac) and *P. citriputeale* (the citrus blast organism) ; it is suggested that all these bacteria may be identical.

* Wilson points out that " The possessive *cerasi*, is preferable to *cerasus* ", hence the change in the last syllable of the specific epithet.

Rosen and Bleecher (10) in a study of pathogenic fluorescent bacteria, including a " pear blast " organism, conclude that " *P. prunicola* can hardly be distinguished from *P. Syringae* and from the pear blast pathogene either in pathogenicity tests or in cultural reactions, and the serological tests further emphasize its relationship to this group " ; they state further that these three organisms " produce symptoms on pear twigs and leaves that are indistinguishable ". These conclusions have recently been confirmed by Wilson (15). The pear disease described by these workers has features in common with a bacterial blossom wilt of pear trees in Britain at present under investigation at East Malling ; this appears to be caused by an organism which in culture is found to have affinity with *P. prunicola* and may prove to be identical with it. The organism was isolated in 1929 from blighted flowering spurs (20) and again, in 1936, not only from spurs but also from fruit spots on pears.

In the hands of various investigators it has been found that, by artificial inoculation, *P. Syringae* can be induced to infect a number of host plants, and recently Rosen (9) has described " a disease of roses involving receptacles, calyx lobes, pedicels and petioles " caused by *P. Syringae*. This organism has been found on lilac in Britain (21) and a similar " blight " of *Forsythia* in this country is probably caused by the same bacterium. If it is eventually found that *P. prunicola* and *P. Syringae* are one and the same organism and can infect all these host plants under field conditions the possibility of inter-infection between ornamental bushes and various species of fruit trees is a point of practical importance not only in nurseries where these host plants may be growing in close proximity but also in mixed fruit plantations.

COMPARISON OF CHERRY BACTERIOSIS WITH THAT OF OTHER SPECIES OF PRUNUS.

The various symptoms of bacteriosis in cherry trees have much in common with those in plum trees. Cankers on stems and branches, shoot wilt, leaf spots and fruit spots are found on both hosts. As already mentioned, gummosis is a conspicuous feature of bacterial cankers in cherries. In plums the exudation of gum on the surface of the lesions is infrequent and only in one variety, Bradley's King damson, has gummosis been found at all comparable with that in cherries. Again, in cherries, the cankering of branches is more common than in plums ; the dying back of individual branches is exceptional in plum trees, even in plantations where stem cankers are numerous.

Foliage infection is more serious, on the whole, in cherries than in plums ; marginal scorching and complete withering of leaves, as described above for cherries, has not been observed in plums.

The sequence of infection in plums and cherries is similar. In both, the stem lesions develop during autumn, winter and early spring ; the organisms in such lesions die during the summer ; but leaf and shoot infections occur in

spring and summer and the leaf spots are probably the sources of infection for stems and branches in the autumn.

In both plum and cherry trees in Britain the organism most generally associated with infections is *P. mors-prunorum* which has been isolated from lesions on stems, branches, shoots, leaf spots and fruit spots on both hosts. In cherries *P. prunicola* also has been isolated from lesions on all these organs, while in plums it has been isolated from green shoots but not yet from cankers on the woody branches and stems.

Again, organisms which in their cultural characters conform to *P. mors-prunorum* rather than to *P. prunicola* have been isolated from peach (stem), myrobolan or cherry plum (stem, branch, leaf), Morello cherry (branch), almond (stem, shoot, leaf) and *Prunus Pissardii* (stem).

A Consideration of Control Measures.

Bacterial diseases of trees, as instanced by Fire-blight of pear and apple trees in America and elsewhere, and walnut Blight, are notoriously difficult to control. Bacteriosis in stone fruit trees is also proving a serious problem, although from a knowledge of the habits of the organisms in the field, methods of control are being sought along several lines.

In the first place mechanical injuries such as pruning and pinching back should be avoided as far as possible during autumn, winter and early spring when stems and branches are more susceptible to infection than in summer. Lesions on stems and branches are inconspicuous during winter and so are difficult of detection. They do not become noticeable until the trees resume growth in spring, and it may then be found necessary to cut out diseased parts, not only to prevent possible further extension of the cankers, but also to remove tissues containing bacteria which may be carried by rain on to the opening leaves to cause leaf spots. This excision of infected parts should be carried out early in the year, as soon as the lesions can be detected ; if left until later the operation is useless for, as already mentioned, the organisms in the cankers die out during the summer and most cankers which have not girdled by mid-summer tend to become healed without treatment. Any open wounds left by cutting out infected parts should be covered with an antiseptic dressing. In America good results are reported against Fire-blight by dressing the cuts and sterilizing the tools with a mercury-glycerine disinfectant, while a zinc chloride preparation proved effective when applied to cankers without excision (5). Whether such treatment would be effective against bacteriosis in cherries is not known but it merits trial.

From field observations and results of inoculation experiments it is assumed that the branches and stems become infected in autumn, and the leaves in spring and summer. Attempts to control the disease by spraying have been directed

therefore to applications to branches and stems in autumn.   Bordeaux mixture
has been recommended for trial, for copper sulphate has been found to be
toxic to the organisms at very low concentrations.   Certain growers have
reported that they have obtained some measure of success in this way, although
controlled spraying trials have not yet given satisfactory results.   If Bordeaux
mixture is applied for this purpose care should be exercised to coat thoroughly
branches, crotch and stem with the spray.

Spraying the foliage has given some degree of control for the leaf spot
phase, but certain varieties of cherries are subject to spray injury from Bordeaux
mixture and the method cannot be recommended for general use.   Thus, in
certain spraying trials at the East Malling Research Station, Bordeaux mixture
caused a bronzing of the foliage and early leaf fall on the varieties Napoleon,
Early Rivers and Bigarreau de Schrecken, while Frogmore was more resistant
to the treatment.*

" Framework grafting ", using resistant varieties for the stems and main
branches, is being investigated as a possible means of controlling Bacterial
Canker in plum trees.   There is hope that for cherry trees, too, resistant stocks
will be found on which the more valuable, but more susceptible varieties may
be worked.   In a plantation in Sussex it was seen that on certain trees, high-
worked on seedling stocks, the scions were killed right down to the union, with
no extension of infection into the stocks, while on other trees the lesions had
extended from the scions into the stocks.   This suggested that stocks may vary
in susceptibility as much as, or possibly more than, scion varieties.   At East
Malling a start has been made in raising resistant stems for frameworking the
more susceptible varieties of cherry, using one of the Devonshire Mazzards
which appear to be the most resistant group in the variety collection.

It has been suggested that the application of lime to the soil might make
trees more resistant to Bacterial Canker and (in cherries) the consequent
gummosis.   Experiments with cherries to test the value of liming for this disease
have not yet been attempted, but in certain trials with young plum trees it
was found that on plots receiving dressings of lime stem cankers resulting from
inoculation with *Pseudomonas mors-prunorum* were, on the whole, greater than
on plots not receiving lime.   It is to be noted in this connection that some of
the outbreaks of Bacterial Canker in Kent are in plantations on or near the
chalk Downs.   Moreover, Mr. N. H. Grubb informs the writer that, in 1926,
he saw, in the Wyre Forest, near Bewdley, Worcestershire, fine healthy cherry
orchards on very acid soil, where bracken is abundant and heather occurs
commonly.   It would seem doubtful, therefore, whether control of Bacterial
Canker is to be obtained by applying lime to the soil.

* Observations by Mr. M. H. Moore.

ACKNOWLEDGMENTS.

SUMMARY.

1. The serious damage caused by bacterial infection in a number of commercial cherry orchards is described.

2. The symptoms of bacteriosis in sweet cherry trees are, stem, crotch and branch cankers, shoot wilt, bud and spur blight, leaf spots, and, occasionally, fruit spots.

3. Certain varieties such as Bigarreau de Schrecken, Cluster Black Heart, Black Tartarian, Emperor Francis, Bradbourne Black and Black Eagle, have proved to be very commonly attacked. Frogmore and Governor Wood, in the observations recorded, have suffered less than other varieties.

4. The organisms causing bacteriosis in sweet cherry trees in Britain are *Pseudomonas prunicola* and *P. mors-prunorum.*

5. A summary is given of the work on bacterial diseases of cherry trees abroad.

6. Cherry bacteriosis is compared with that on other species of *Prunus.*

7. Methods for controlling these bacterial diseases in cherry trees are discussed.

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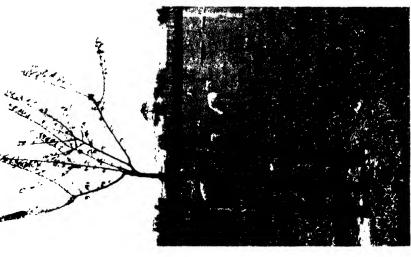
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Fig. 1.

Healthy cherry tree of same age as tree shown in Fig. 2

Fig. 2
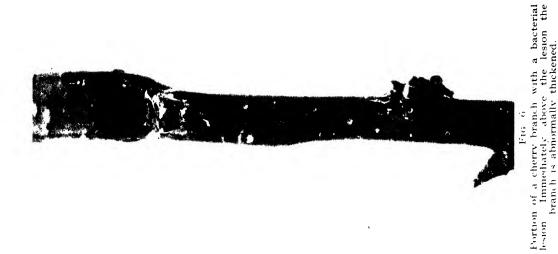
Cherry tree (three years from planting) with Bacterial Canker on the stem.

Fig. 3.

Cherry tree (three years from planting) with branch lesions.

FIG. 4

A young cherry tree with lesion on stem. Note the dead buds on the lesion and the curled leaves above it, as compared with the flattened leaves below.

FIG. 5

The tree shown in Fig. 4 as seen a few weeks later. Above the canker the leaves have wilted; below, the buds have grown out to form strong shoots.

FIG. 6

Portion of a cherry branch with a bacterial lesion. Immediately above the lesion the branch is abnormally thickened.

FIG. 9.
Bacterial lesion on shoot, killing terminal portion.
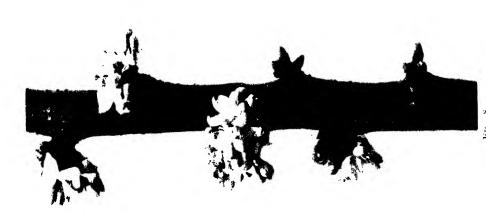
FIG. 8
Spur infections through buds

FIG. 7.
Nodal infection through buds.

FIG. 10

Severe leaf infection causing withering of foliage,
var. Cluster Black Heart

FIG. 11

Leaf infection : rather large spots more or less isolated,
var. Black Tartarian

FIG. 12.

Leaf with small crowded spots;
var. Black Eagle.

FIG. 13.

Young cherry leaves showing infection extending
from petiole into the midrib.

OBSERVATIONS ON THE EFFECT OF POTASH SUPPLY ON THE WATER RELATIONS OF APPLE TREES

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In the absence of an adequate supply of potash the leaves of apple trees and of many other plants develop symptoms usually associated with a deficient water supply (1, 4). It appears that an abundant supply of potash is necessary for the maintenance of a favourable water balance in the foliage. The exact role which the potash plays is not known. The maintenance of a favourable water balance in the foliage demands that loss of water by transpiration from the leaves shall not, except for short periods, exceed the supply of water to them through the shoot. It seemed desirable, therefore, to investigate the effect of potash supply on (1) the resistance offered to the flow of water to the leafy shoots, and (2) the transpiring surface, and to determine whether the ratio resistance/transpiring surface is affected by the level of potash supply.

Measurements of the resistance offered by shoots to the flow of water have therefore been made and a rough approximation to the transpiring surface of the leafy shoots has been obtained. An accurate determination of transpiring surface is not possible, but a first approach to the problem seems to be a determination of leaf areas supported by data for stomatal frequency. This has been done and it has been shown from what follows that the ability of cut shoots to transmit water when compared with the area of leaves to be supplied, is lower in material from trees with a deficient potash supply than in material from trees in which the supply of potash is adequate.

EXPERIMENTAL.

I. MATERIAL.

Material from two sources was used in the investigation.

(a) *Long Ashton.* Shoots were obtained from two sets of trees receiving different manurial treatment. The trees were of the variety Lane's Prince Albert, on Malling No. II rootstock. They were planted as maidens in December 1926, and since that date have been under a system of arable cultivation.

Manures have been applied each year as under :—

		Complete fertilizer plot. *cwts. / acre.*	*No Potash plot.* *cwts. / acre.*
Nitrate of Soda	..	2	2
Superphosphate	..	3	3
Sulphate of Potash	..	3	—

These will be referred to as " potash " and " no potash " material respectively.

There was here a large and obvious response to the potash, the no potash trees being much smaller than those receiving potash and exhibiting typical symptoms of potash starvation.

(b) *Goostrey (Cheshire)*. Shoots here were obtained from two sets of trees of the variety Bramley's Seedling, on Crab rootstock, planted as 3-year-old trees in the winter 1919-20. One plot has received no manure and the other 3 cwt. of sulphate of potash per acre each year for the last six years. No nitrogen or phosphate has been supplied. As the trees are under a system of arable cultivation, nitrogen supplies are likely to have been adequate. No signs of phosphate deficiency are evident and it is extremely unlikely that any such deficiency exists.

2. METHODS.

Comparable leader shoots were taken during July and August from two sets of trees, cutting at the base of the previous year's growth. Only shoots not under the direct influence of fruit were used. The shoots were removed to the laboratory at once and the following determinations made :—

(1) *Length of growth in current year*. This gives an approximate measure of the response to potash.

(2) *Number of leaves per shoot*.

(3) *Leaf area per shoot*. All the leaves on the selected shoot were removed and tracings made of them on paper of good quality. The leaf tracings were cut out and weighed and the total area of foliage carried by each shoot was calculated.

(4) *Stomatal frequency*. It was not practicable to peel off the lower epidermis to make stomatal counts. It proved more convenient to scrape the lower surface of the leaf with a sharp scalpel, and to mount the scrapings for examination. For each leaf about thirty microscope fields were counted.

(5) *Conductivity of shoots*. For these determinations a portion of the distal end of the previous year's shoot, about 15 cm. in length, was

selected, so that it was possible to measure the resistance to the flow of water to the shoot of the current year's growth. Pieces of stem 15 cm. in length were cut and placed in recently boiled and cooled distilled water and the vessel of water with the pieces of shoot in it kept under reduced pressure until no further air bubbles escaped from the shoots. Release of the diminished pressure caused injection of the shoots by the water.
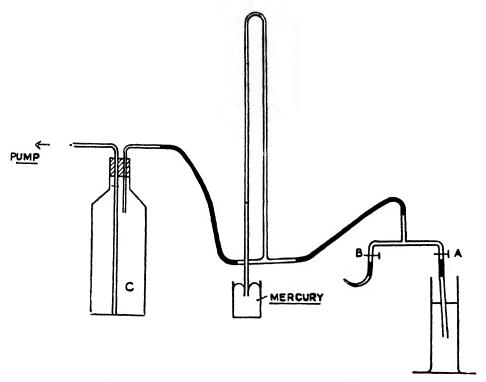
FIG. 1. Apparatus used for determining Water Conductivity of Shoots.
For explanation see text.

Measurements of the ability of these pieces of shoots to transmit water were made with the apparatus illustrated. The tap A was closed, and by suitable adjustment of tap B connected to a capillary, constancy of pressure was attainable. A pressure of about 30 cm. of mercury was employed. During an experiment, provided a fairly large reservoir of air (C) was in circuit, variations in pressure usually amounted to only 2-5 mm. (i.e. possible error of about 1·5%). When greater variations than this were obtained the experiment was discarded. The shoots were fixed firmly to the tube connected with tap A, with the lower end of the shoot immersed in a weighed cylinder of water. Tap A was then opened and suction applied for about ten minutes, tap B remaining open meanwhile. The cylinder was then re-weighed and the difference between

this and the previous weight gave the amount of water that had passed through the shoot. From these data a figure called the " actual conductivity " was calculated. This is the amount of water which would pass through a 15 cm. length of stem in 15 mins. with a pressure of 30 cm. of mercury. These units, of length, time and pressure, were chosen as Farmer (2) employed them in his determinations of specific conductivities of the woods of evergreen and deciduous trees. It was assumed for the purpose of calculation that the water transmitted is directly proportional to time and pressure.

RESULTS.

The data obtained are incorporated in Table I. Mean values are given, together with the standard errors of the mean.

TABLE I.

| | Long Ashton Material. | | Goostrey Material. | |
	No Potash.	Potash. (Mean Values ±	No Potash. Standard errors).	Potash.
Number of Shoots	21	14	11	12
Length of Shoot cm.	12·9 ± (1·51)	36·9 ± (2·26)	16·7 ± (1·77)	24·1 ± (1·21)
No. of leaves per shoot	11·9 ± (0·92)	19·4 ± (0·95)	14·6 ± (0·83)	16·6 ± (0·47)
Leaf area per shoot cm.^{2}	218·4 ±(16·9)	419·6 ±(29·8)	466·6 ±(24·0)	548·5 ±(34·0)
Stomatal frequency per mm.^{2} ..	478·8 ±(13·2)	382·3 ±(10·7)	329·2 ± (9·82)	282·0 ± (8·64)
Actual conductivity gm. H$_2$O ..	1·94± (0·366)	5·36± (0·311)	6·74± (0·61)	8·33± (0·53)

From these data certain other values have been calculated and these are tabulated below.

TABLE II.

| | Long Ashton. | | Goostrey. | |
	No Potash.	Potash.	No Potash.	Potash.
Mean area of leaf (cm.^{2})	18·35	21·58	31·91	33·04
Number of stomata per leaf, in 1,000's ..	871	825	1,050	932
Number of stomata per shoot in 1,000's ..	10,370	16,041	15,360	15,468
Conductivity, gm. per 100 cm.^{2} leaf	0·83 ± (0·107)	1·32 ±(0·077)	1·47 ±(0·136)	1·53 ±(0·170)
Conductivity, gm. per 10^6 stomata	0·175	0·345	0·446	0·543

DISCUSSION.

Reference to Table I shows that for the Long Ashton material the values obtained in each case for potash and no potash material differ significantly. The response to potash is reflected in the increased shoot growth, the length of the current year's shoots being increased by almost 200%, whilst the total leaf area per shoot is almost doubled. Stomatal frequency is significantly lower in the potash material. The conductivity of the shoots differs markedly. The larger shoots of the potash material transmit a much greater amount of water than the no potash shoots.

The results for the material from Goostrey are similar, but the differences here are much smaller and often only just reach or nearly reach the level of significance.

Turning to Table II, it is seen that potash has increased the mean leaf size, and with this is to be correlated the decreased stomatal frequency. For Seakale beet (6) it has previously been shown that increased potash supply results in the development of larger leaves, the increase in leaf size being effected through an increase in cell size and accompanied by a decrease in stomatal frequency. The number of stomata per leaf is unaltered. A similar result has been obtained here. It is possible here to obtain a calculated value for the total number of stomata per leaf, and slightly fewer stomata are found in the larger leaf of the potash material than in the smaller leaf from the no potash shoots. Having regard to the method of calculating this figure, no significance can be attached to the difference.

The calculated statistics referring to the relation between the water transmitted to the shoot and the leaf area indicate a relatively deficient supply of water to the foliage of the no potash material. If the basis of expression instead of being leaf area is number of stomata, the no potash material occupies a still more unfavourable position. Under the stated conditions of length, time and pressure, in the no potash material, 0.175 gm. of water is transmitted per million stomata as compared with 0.345 gm. for the potash material. This refers to the material for Long Ashton. For the Goostrey trees, where potash deficiency is less severe, the difference is smaller but in the same direction.

The increased stomatal frequency in the no potash material can probably be correlated with the higher transpiration rates found by Mann (3) for the foliage of potash-deficient apple trees under conditions of abundant water supply and high light intensity. If the water supply is near deficiency point, it is to be expected that the leaves of the no potash material will feel the effects of the deficiency before the foliage of plants receiving a full potash supply, due in part to the greater stomatal frequency. Owing to the lower transmitting power of the shoots of the no potash material, a deficient supply to the leaves may

occur earlier than with the potash material. When the supply of water to the roots is abundant this may be of little importance. With a full water supply the relatively inefficient shoot of the no potash trees may be able to transmit sufficient water for the requirements of the foliage. With a lowered water supply to the roots, it seems likely that only the efficient shoots of the potash material can maintain a favourable water balance in the leaves. Hence it is to be expected that potash deficient trees will show signs of water shortage earlier than trees with a full potash supply, which is in accordance with field observations. The data presented, too, accord with observations of Wallace (5) that apple trees receiving a small supply of potash show much less evidence of potash starvation when the water supply is maintained at a high level. Abundant water mitigates certain effects of potash starvation.

The relative inefficiency of the shoots of the no potash material might be of less importance if a greater force were available for overcoming the resistance to the flow of water. Such data as are available, however, indicate that the osmotic pressure of sap expressed from the tissues (and hence probably the maximum suction pressure of the cells) is lowered when the potash supply is :reduced (6).

It is concluded, therefore, that one effect of a deficient supply of potash to apple trees is to reduce the conductivity of the shoots. The conductivity is reduced to a greater extent than leaf area is reduced, so that the ratio conductivity of shoot/leaf area to be supplied, is lowered when the supply of potash is deficient. Owing to the increased stomatal frequency, a still greater reduction occurs in the ratio conductivity of shoot/number of stomata to be supplied.

This, it is suggested, is one of the reasons why potash-deficient apple trees are extremely susceptible to the effects of water shortage.

SUMMARY.

1. Observations have been made on the conductivity (of shoots), leaf area and stomatal frequency, of shoots of apple trees receiving a full supply of potash and of trees whose supply of potash is deficient.

2. Material from two sources was used in the investigation.

3. It is shown that the effect of potash deficiency is to
    (a) significantly decrease shoot length ;
    (b)   ,,   ,,   number of leaves per shoot ;
    (c)   ,,   ,,   area of foliage carried by the shoot ;
    (d)   ,,   ,,   the ability of the shoot to transmit water ;
    (e)   ,,   increase stomatal frequency ;
    (f)   ,,   decrease the ratio conductivity/leaf area ;
    (g) decrease (probably significantly) the ratio conductivity/number of stomata.

4. It is suggested that the reduction in the ratios conductivity/leaf area and conductivity/number of stomata is one of the reasons why potash deficient trees are so susceptible to drought conditions.

ACKNOWLEDGMENTS.

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INVESTIGATIONS ON EGG-KILLING WASHES

II. THE OVICIDAL PROPERTIES OF HYDROCARBON OILS ON *APHIS POMI* DE GEER

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THE experiments of Tutin (9) on the effect of tar oils on the eggs of the Winter Moth, *Cheimatobia brumata* L., lead to the hypothesis, put forward by Staniland, Tutin and Walton (7), that ovicidal action is due to the physical stifling action of the oil deposit. Provided that the chemical stability of tar oils is kept constant, ovicidal efficiency should increase, up to a point, with increase in the boiling ranges of the oils. This conclusion has been verified in so far as tar oils are concerned by the further field work of the Long Ashton investigators on the control of the capsid bugs *Lygus pabulinus* L. and *Plesiocoris rugicollis* Fall. and by the latter work of Tomaszewski and Fischer (8) on the eggs of the silkworm, Winter Moth (*C. brumata*) and Apple Sucker (*Psyllia mali* Schm.). Further, the hypothesis has been confirmed experimentally for hydrocarbon oils of mineral origin, in particular by Austin, Jary and Martin (1), using eggs of *L. pabulinus* as test material. The evidence assembled by Martin (5) is unanimous in indicating that the insecticidal properties, in general, of petroleum oils increases with the boiling range of the oil until maximum efficiency is reached. The characteristics of a hydrocarbon oil suitable for the control of Capsid Bug or Winter Moth at the egg stage may, therefore, be regarded as known.

Petroleum oils of the usual types fail, however, to inhibit the hatching of aphis and psyllid eggs. Staniland, Tutin and Walton, therefore, suggested that ovicidal action on such eggs is associated with a chemical toxicity possessed by tar oils but not, in general, by petroleum oils. From this hypothesis it does not follow axiomatically that chemical stability and non-volatility are important factors in determining the toxicity of tar oils to aphis and psyllid eggs. Tutin stated, however, that in preliminary experiments with eggs of *Aphis pomi* De Geer, high-boiling tar oils were found more toxic than low-boiling oils, though he did not quote experimental results in support of this statement. Hartzell and Parrott (3), from critical studies on eggs of a number of insect species, were unable to substantiate Tutin's statement, for, although certain of their results were explainable upon this hypothesis, their tests on eggs of *A. pomi* indicated that relatively low-boiling tar oils are highly toxic. For this reason and because of the low boiling ranges of certain commercial preparations used

with apparent success on the Continent, Martin (5) included in the standard specifications which he suggested for consideration by manufacturers a Grade B oil of relatively low boiling range for use when only chemical toxicity to aphis and psyllid eggs was required of the oil. Because of this discordance, the primary purpose of the work to be described below was the examination of the comparative efficiencies of high and low boiling oils as ovicides against *A. pomi*.

A second object of the work has been the application of the dimethyl sulphate solubility test to the differentiation of those hydrocarbon oils most effective against aphis and psyllid eggs. The broad distinction that petroleum oils are ineffective whereas tar oils are effective as toxic ovicides lacks precision, and an analytical method is required which will evaluate toxic properties. Petroleum and neutral tar oils are both hydrocarbon oils differing in that the former are relatively poor whereas the latter are rich in aromatic hydrocarbons. Austin, Jary and Martin (1) accordingly used the Valenta test for the separation of aromatic hydrocarbons by virtue of their solubility in dimethyl sulphate. They found that this test successfully indicated those of a number of oils which were effective in the field in the control of *Psyllia mali*. It was considered necessary to substantiate this observation by laboratory tests upon aphis eggs.

Since the start of this work, Tomaszewski and Fischer (8) have published the results of their trials in which, among other factors, the influences of boiling range of tar oils and their solubility in dimethyl sulphate on the toxicity to eggs of *P. mali* were investigated. Unfortunately these authors gave no data by which the probable error of their results can be deduced and, although their results undoubtedly point to the greater toxicity of oils of high boiling range, it is impossible to trace any marked influence of the dimethyl sulphate solubility figure. It would seem, from their results with a given oil at different concentrations, that the field method they used demonstrated only large differences in toxicity.

Finally, an attempt has been made to determine the particular compound or types of compound to which the toxicity of tar oils to eggs of *A. pomi* is due.

EXPERIMENTAL METHODS.

Permanent Apple Aphis (*A. pomi*), was used as the test insect, as this species is the only one of the fruit aphides that can be reared successfully to lay eggs in large numbers under controlled conditions. Further, this species is more readily handled in the laboratory than others ; in particular, the mode of egg laying permits of the easy removal of the eggs from bark for the purpose of counting. The eggs of other species, such as *Anuraphis roseus*, are much more sparsely distributed and it would necessitate the treatment and examination of a very considerable number of shoots in order to obtain the number of eggs

required for each test.   Field experience has shown that the eggs of the various fruit tree aphides exhibit susceptibilities of the same order to tar oil washes.

Shoots bearing eggs were cut during dry weather in winter and taken to the laboratory.   They were carefully selected so that each had several hundred eggs of normal and uniform appearance.   Not less than four shoots were used for each individual test and they were dipped into the insecticide, care being taken to agitate the fluid so that any air film on the shoots or around the eggs was broken.   The shoots remained in the fluid for 15 seconds.   After treatment they were attached to a strip of wood so arranged that the formation of areas of high concentration of the wash during drying was avoided.   The basal ends of the dried shoots were cut off and rejected and the cut ends thrust into damp sand in pots.   The pots were allowed to remain out of doors, exposed to the weather, until the first few eggs on the untreated controls showed signs of hatching, which usually occurred 4-10 days after the outer egg membrane had split longitudinally.   The shoots were then taken into the laboratory and eight samples of each treatment (lengths of $1\frac{1}{2}$-$2\frac{1}{2}$ in.) were cut at random.   Each of these lengths was impaled on a pin held by a cork inserted into an open-ended tube of $5 \times \frac{5}{8}$ inch dimensions.   The open end was afterwards plugged with cotton wool.   During the above sampling the twigs were handled in such a manner that none of the eggs was damaged.   The tubes were arranged in trays to facilitate inspection.

In most instances the hatched nymphs wandered away from the pieces of shoot and in due course died in the tube.   The cotton wool plug permitted the shoot and aphides to dry out without decomposition or development of moulds.

The total number of eggs on each shoot was counted after removal of the eggs by gently heating the shoot in 10% caustic soda in a water bath for about 10 minutes.   Care was taken to avoid decomposition of the bark by excess heating. The eggs were filtered off, washed with weak acid and counted in 5-inch Petri dishes over squared paper.   The aphides were readily counted by shaking and brushing them into a Petri dish.   On each piece of shoot there were not less than 100 eggs and in most cases there were 300.

In the 1935 trials, the stock of aphis eggs was obtained from the new wood on an apple stool bed.   The bed was carefully sprayed in August to remove all species of Hemiptera.   The shoots were then infested with colonies of Permanent Apple Aphis, which bred very rapidly on the vigorous new growth and laid numerous eggs during October.

In the 1936 trials, the stool bed material failed, owing to unfavourable weather conditions, and material was therefore collected from a number of Worcester Pearmain nursery trees, considerable care being taken to collect uniform material.

In both seasons the treatments were carried out during the last week of January ; hatching began at the end of March and was completed by mid-April. The majority of the eggs hatched during the first week of April.

Parasitism of the eggs was not observed during the two seasons' experiments. Observations were made on the influence of the condition of the shoot on the viability of the eggs. It was found that eggs laid on weak shoots and stools, or on shoots covered with apple Scab lesions were frequently of low viability. In the experiments care was taken to eliminate shoots of this type.

MATERIALS USED.

Biological laboratory tests of the materials were carried out during two seasons, 1935-6. The oils and preparations are designated in the tables by letter and number, the tar oils are identified by the letter T, the petroleum oils by P, and the commercial preparations by C.

Representative samples of Grade A tar oils were employed together with oils of lower boiling range, certain of which conformed to the requirements of Grade B specification. The results obtained when these oils were subjected to analysis by the methods described by Martin (5) are recorded in Table I. The oils T.10 and T.11 are both Grade A oils ; T.12 and T.13 come within the limits proposed for Grade B oils except that T.12 contains a small percentage of neutral oils, insoluble in dimethyl sulphate, whereas T.13 is of slightly low specific gravity. T.14, a low-boiling creosote oil, is perhaps non-typical in that the tar acid content is abnormally low. The oil T.13 was therefore fractionated into a high boiling fraction, T.13a, so selected that the oil remained liquid on cooling, and a low boiling fraction, T.13b. Although anthracenoid solids were deposited from the neutral oils of T.13a, no difficulty was met with during the preparation and application of the ovicide.

In connection with the work on the identification of the compounds responsible for the toxicity of tar oils to aphis eggs, the neutral oils of T.11 were treated for the removal of oxygenated derivatives. As the physical constants of the untreated neutral oil, which was given the code number T.11a, are those quoted for T.11, neither this nor the treated oil T.11b, appears in Table I.

In addition to the tar oils, a number of commercial preparations were included in the tests. C.1 and C.2 are samples of a winter wash preparation widely used by growers to whom it is described as a tar oil wash. This preparation is of the miscible oil type, whereas C.3 is a sample of a stock emulsion also widely used by growers. The latter produce is guaranteed by the makers to satisfy the requirements of the Grade A. Type S.E. specification. The sample C.4 is of a proprietary disinfectant submitted by the makers with particulars of composition, for an opinion of its merits as a fruit tree wash. On the analytical

evidence alone it was considered unsuitable for such a use, but the material was included in the tests because of the low boiling range of the neutral oils, its relatively high content of tar acids and its interest for comparison with T.14. The products C.5 and C.6 are samples of experimental stock emulsions compounded from hydrogenated tar oils.   Hydrogenation has apparently effected a reduction of part of the aromatic derivatives to hydrocarbons insoluble in dimethyl sulphate. Analysis of these products gave no difficulty except that, with C.6, the fraction of neutral oil insoluble in dimethyl sulphate separated as a yellow solid which, on standing, ultimately formed a liquid layer the volume of which could be determined.   A check determination was carried out with ethylene glycol monoacetate, a preferential solvent suggested by Edwards and Lacey (2) for the separation of high-boiling tar and petroleum oils ; 19 per cent. by volume of the neutral oils proved insoluble in this reagent.   A further point of interest met in the analysis of these products was the evolution of hydrochloric acid gas during the initial stages of the determination of the boiling ranges of the neutral oils of both products.   It would appear that, during the extraction of tar bases with dilute hydrochloric acid, oxonium compounds are formed which, though stable enough to withstand the removal of solvent, are decomposed at the higher temperatures required for distillation.

TABLE I.

Analysis of tar oils and preparations.

| | % by weight. | | | Neutral Oils. | | | | | |
| | | | | | Boiling Range. | | | | |
	Neutral oil.	Tar acids.	Tar bases.	Sp. Gr. (60° F.)	10% by vol.	50% by vol.	80% by vol.	Unsulphonated residue : % by vol.	Dimethyl sulphate : % by vol. insoluble in
T.10	89·4	4·1	5·4	1·063	274	337	390	—	0
T.11	90·6	4·0	—	1·104	267	324	365	—	0
T.11a	100·0	0·0	0·0	—	—	—	—	—	—
T.11b	100·0	0·0	0·0	—	—	—	—	—	—
T.12	86·1	8·4	4·7	1·049	253	306	352	—	6
T.13	87·4	10·7	4·0	1·040	254	307	349	—	0
T.13a	92·4	8·6	5·4	1·066	298	333	372	—	7
T.13b	85·9	10·7	4·0	1·009	234	260	292	—	4
T.14	91·8	2·3	4·0	0·974	203	234	276	—	11·5
C.1	62·4	8·1	2·0	1·010	243	320	376	—	33
C.2	64·6	8·4	3·4	1·002	249	310	363	—	31·5
C.3	63·2	2·5	3·5	1·079	280	330	371	—	0
C.4	30·6	19·8	1·2	1·026	222	246	300	—	0
C.5	68·2	1·3	2·5	1·036	329	371	400	12	36
C.6	67·5	1·3	2·1	1·073	335	365	400	—	22

Table II.

Results of biological tests of tar oils and preparations.

Test Insect—*A. pomi.*

Year of Test		1935.		1936.	
Code letter, number and concentration of material.		No. of eggs in test.	Mean per cent. hatch.	No. of eggs in test	Mean per cent. hatch.
	per cent.				
T.10	2·0	1,545	9·5 ± 4·52		
T.10	1·0	3,313	44·6 ± 4·49		
T.11	2·0	—	—	2,621	2·7 ± 0·87
T.11	1·5	—	—	2,990	10·7 ± 2·32
T.11a	2·0	—	—	1,686	3·8 ± 1·62
T.11a	1·5	—	—	1,976	6·8 ± 2·04
T.11b	1·5	—	—	3,316	5·4 ± 0·97
T.12	2·0	3,432	26·9 ± 3·76		
T.12	1·0	1,767	54·7 ± 6·25		
T.13	2·0	1,672	18·9 ± 4·18	2,234	80·2 ± 3·16
T.13	1·5			2,506	56·9 ± 4·12
T.13	1·0	1,088	34·2 ± 12·74		
T.13a	2·0	1,630	1·0 ± 0·42		
T.13b	2·0	1,436	60·8 ± 3·85		
T.14	2·0	2,268	21·8 ± 4·79		
T.14	1·0	1,835	63·1 ± 8·28		
C.1	4·0	1,689	20·9 ± 4·99		
C.1	2·0	2,647	40·9 ± 8·26		
C.2	3·0	—	—	1,837	12·8 ± 2·88
C.3	3·0	—	—	1,546	2·24 ± 0·90
C.4	4·0	1,456	57·7 ± 7·45		
C.4	2·0	1,332	33·2 ± 7·94		
C.5	3·4	—	—	2,084	3·7 ± 2·00
C.6	3·0	—	—	1,842	0·0 ± 0·00
Soap + benzene	0·7 } 2·5 }	1,940	70·9 ± 4·89	1,934	79·5 ± 3·37
(1) Soap	0·7	1,876	54·3 ± 8·52		
(2) Soap	0·7	1,378	40·7 ± 5·17		
Untreated		1,423	53·8 ± 5·39	3,481	96·1 ± 1·49

For the test of oils rich in constituents insoluble in dimethyl sulphate, a number of petroleum oils and preparations were used, the analyses of which are given in Table III. P.19 and P.4b were samples of light lubricating oils, the former being a highly-refined oil, the latter being a semi-refined spindle oil. The preparation C.7, compounded from an oil similar in characteristics to P.4b, was of the stock emulsion type. The remaining two petroleum oils were residues from petroleum oil refinement, P.18 being a high-boiling sulphur dioxide soluble fraction isolated by the Edeleanu process. This oil contains aromatic hydrocarbons, for 23% of the neutral oils are soluble in dimethyl sulphate. The oil P.20, of which a complete chemical examination was not carried out, was included because it proved completely soluble in dimethyl sulphate, but the predominant constituents are sulphur derivatives and not hydrocarbons.

TABLE III.

Analysis of petroleum oils and preparations.

| | % by weight. | | | | Neutral Oils. | | | | |
| | | | | | Boiling Range. | | | | |
	Neutral oils.	Alkali sol. (Tar acids).	Acid sol. (Tar bases).	Sp. Gr. (60° F.).	10% by vol.	50% by vol.	80% by vol.	Unsulphonated residue: % by vol.	Dimethyl sulphate: % by vol. insoluble in
P.4b	100	0·0	0·0	0·989	348	378	394	74	100
P.18	96·2	2·6	0·3	1·003	320	359	394	14	77
P.19	100	0·0	0·0	0·860	312	347	369	98	100
P.20	99·6	0·4	0·0	0·962	181	203	264	—	0
C.7	62·0	0·0	0·0	0·883	347	382	—	76	100

TABLE IV.

Results of biological tests of petroleum oils and preparations.

Test Insect—*A. pomi.*

Year of Test		1935.		1936.	
Code letter, number and concentration of material.		No. of eggs in test.	Mean per cent. hatch.	No of eggs in test.	Mean per cent. hatch.
	per cent.				
P.4b	5·0			2,009	35·4 ± 8·92
P.18	8·75	1,064	16·9 ± 5·84		
P.18	6·5			1,688	26·6 ± 5·14
P.18	4·35	2,266	59·8 ± 5·16		
P.19	5·0	1,167	43·5 ± 7·18		
P.20	2·0	1,011	39·6 ± 9·16		
P.20	1·0	1,428	43·9 ± 2·83		
C.7	8·3	1,524	35·4 ± 5·53	2,334	31·9 ± 5·03
Soap + benzene	0·7⎫ 2·5⎭	1,940	70·9 ± 4·89	1,934	79·5 ± 3·37
(1) Soap	0·7	1,876	54·2 ± 8·52		
(2) Soap	0·7	1,378	40·7 ± 5·17		
Untreated		1,423	53·8 ± 5·39	3,481	96·1 ± 1·49

For the preparation of the ovicides, the oils were emulsified by the two-solution oleic acid method (4), each wash containing, in addition to the required amount of oil, 0·6% oleic acid and 0·1% sodium hydroxide. When solid hydrocarbons were tested, the required weight was dissolved in benzene and to the solution the oleic acid was added, this mixture then being poured into the dilute sodium hydroxide solution completing the final volume of the wash. The amount of benzene used was sufficient to give a final concentration of

2·5% by volume.   For check purposes, soap solutions prepared by the addition, with or without benzene, of oleic acid in dilute sodium hydroxide solutions were used and, in two cases, benzene was added in parallel trials to washes containing hydrocarbons.   From the commercial preparations, washes were prepared by direct addition to distilled water in the proportions stated in Tables II and IV.

DISCUSSION OF RESULTS.

The results of the counts of hatched aphides and total eggs are expressed in Tables II and IV as the mean figure for the percentage hatch of each of the eight (or seven) samples of eggs, together with the standard error of the mean. For simplification of discussion, the results will be considered, as far as possible in connection with the particular point under investigation, namely :—

I. THE INFLUENCE OF BOILING RANGE ON OVICIDAL PROPERTIES.

To eliminate the chemical factors, it is necessary to consider only the results obtained with oils showing a high solubility in dimethyl sulphate (i.e. tar oils).   These results have, therefore, been abstracted to form Table V in which, as an index of the boiling range of the oil, the temperature at which 50% distils over is quoted.

The results show that the ovicidal properties, to *A. pomi*, of the tar oils under consideration increase with boiling range, a conclusion in agreement with Tutin's statement.   At concentrations of 2·0 and 1·5% oils conforming to the Grade A specification have given a lower percentage hatch than oils of lower

TABLE V.

The Influence of Boiling Range on Ovicidal Properties.

	Boiling range 50% distil below.	Mean percentage hatch.		
		2% oil (Total).	1·5% oil (Total).	1% oil (Total).
1935 :				
T.10	337° C	9·5 ± 4·52	—	44·6 ± 4·49
T.13a	333° C.	1·0 ± 0·42	—	—
T.13	307° C.	18·9 ± 4·18	—	34·2 ± 12·74
T.12	300° C.	26·9 ± 3·76	—	54·7 ± 6·25
T.13b	260° C.	60·8 ± 3·85	—	—
T.14	234° C.	21·8 ± 4·79	—	63·1 ± 8·28
Untreated	—	—	53·3 ± 5·29	—
1936 :				
T.11	324° C.	2·7 ± 0·87	10·7 ± 2·32	—
T.13	307° C.	80·2 ± 3·16	56·9 ± 4·12	—
Untreated	—	—	96·1 ± 1·49	—

boiling range.   It is of interest to note that at the highest concentration, these oils have not given complete control, an indication that the concentration of 2·5% neutral oil soluble in dimethyl sulphate (corresponding to approximately 3% Grade A oil), a figure arrived at from general experience in the field as the minimum content of a tar oil wash able to control aphis and sucker, is not excessively high.

II. The Influence of Solubility in Dimethyl Sulphate on Ovicidal Properties.

To eliminate, as far as possible, the effects of boiling range on ovicidal efficiency, the comparison, given in Table VI, is confined to high-boiling tar and petroleum oils.   The temperature at which 50% by volume of the neutral oils distils over is therefore quoted in the table, together with the actual oil concentration used and the estimated content of neutral oils soluble in dimethyl sulphate.   In each year's group, the various ovicides are arranged in order of decreasing content of neutral oils soluble in dimethyl sulphate.   This figure was calculated on the assumption that the specific gravity of oils and preparations is 1·00, an assumption that introduces a slight but negligible error.

Table VI.

The Influence of Dimethyl sulphate Solubility on Ovicidal Properties.

| | | Boiling range 50% distil below. | % Neutral oils. | | Mean percentage hatch. |
			Total.	Sol. in dimethyl sulphate.	
1935 :					
8·75%	P.18	359° C.	8·4	1·9	16·9 ± 5·84
2·0 %	T.10	337° C.	1·8	1·8	9·5 ± 4·52
2·0 %	T.13a	333° C.	1·85	1·7	1·0 ± 0·42
4·0 %	C.1	320° C.	2·5	1·7	20·9 ± 4·99
4·35%	P.18	359° C.	4·2	0·95	59·8 ± 5·16
1·0 %	T.10	337° C.	0·9	0·9	44·6 ± 4·49
2·0 %	C.1	320° C.	1·25	0·85	40·9 ± 8·26
8·3 %	C.7	382° C.	5·2	0·0	35·4 ± 5·53
5·0 %	P.19	347° C.	5·0	0·0	43·5 ± 7·18
1936 :					
2·0 %	T.11a	324° C.	2·0	2·0	3·8 ± 1·62
3·0 %	C.3	330° C.	1·9	1·9	2·24± 0·90
2·0 %	T.11	324° C.	1·8	1·8	2·7 ± 0·87
3·0 %	C.6	365° C.	2·0	1·6	0·0 ± 0·00
3·4·%	C.5	371° C.	2·3	1·5	3·7 ± 2·00
1·5 %	T.11a	324° C.	1·5	1·5	6·8 ± 2·04
6·5 %	P.18	359° C.	6·25	1·43	26·6 ± 5·14
1·5 %	T.11	324° C.	1·36	1·36	10·7 ± 2·32
3·0 %	C.2	310° C.	1·94	1·33	12·8 ± 2·88
5·0 %	P.4b	378° C.	5·0	0·00	35·4 ± 8·92
8·3 %	C.7	382° C.	5·2	0·00	31·9 ± 5·03

It is evident from Table VI that the total concentration of hydrocarbon oils is no criterion of the ovicidal efficiency of the emulsions upon *A. pomi*, and that a high degree of correlation is shown between the ovicidal properties of the washes and their content of neutral oils soluble in dimethyl sulphate. This conclusion involves the assumption that the oils are of a sufficiently high boiling range, for it will be noted that the petroleum residue P.20 (Table IV), although completely soluble in dimethyl sulphate, was an inferior ovicide. At 2% the mean percentage hatch was 39·6 ± 9·16, a figure not significantly different from the hatch on untreated twigs. This failure is to be ascribed to the low boiling range of the oil.

These results afford further evidence that the division of hydrocarbon oils into tar oils and petroleum oils respectively is insufficient to differentiate oils suitable and unsuitable for use as aphis ovicides. The likelihood of the introduction of hydrogenated and low temperature tar oils, which may contain high percentages of oils of a non-aromatic character ; the use, on the Continent, of lignite tar oils which may likewise contain paraffinoid hydrocarbons ; the fact that certain types of high-boiling petroleum oils may contain appreciable amounts of aromatic hydrocarbons ; these three considerations make it necessary to adopt a more precise distinction. Unfortunately, although the term aromatic hydrocarbons goes far to meet requirements, there is no corresponding term to embrace the naphthenic, the unsaturated and the saturated hydrocarbons, which are the predominant constituents of hydrocarbon oils. The best alternative would seem to be the adoption of definitions based on the actual analytical process, even though this introduces the cumbersome expression " neutral oils soluble (or insoluble) in dimethyl sulphate ".

III. THE OVICIDAL CONSTITUENTS OF NEUTRAL OILS SOLUBLE IN DIMETHYL
    SULPHATE.

The suggestion put forward in the above paragraph involves the assumptions that either the individual constituent hydrocarbons of high boiling neutral oils, soluble in dimethyl sulphate, possess similar ovicidal properties to aphis and psyllid eggs, or the relative proportions of these compounds are identical in any sample of these oils. These assumptions can be put to experimental test only with difficulty, for little is known of the actual compounds present in high-boiling hydrocarbon oils, and those compounds which have been isolated appear to be present only in small amount. Two methods of attack were used, the first being the chemical fractionation of the neutral oils and the second the testing of individual compounds. For the former method, the neutral oil was treated, in light petroleum solution, with hydroferrichloric acid (6), which removes certain of the oxygenated derivatives as a sludge. The percentage hatch obtained with this treated oil (T.11b) at 2% was 5·4 ± 0·97, which is not significantly different

from that of the untreated oil, $6 \cdot 8 \pm 2 \cdot 04$.   It may therefore be concluded that the ovicidal constituents are not fractionated by the treatment.

For the trials of individual compounds, a number of hydrocarbons present in low- and high-boiling tar oils were used together with a few miscellaneous products.   Diphenyl oxide, for example, was included because this compound proved the most toxic of the tar oil constituents tested by Staniland, Tutin and Walton (7) on eggs of *C. brumata*.   Tetralin (tetrahydro-naphthalene) and dekalin (decahydronaphthalene) were tested because Woodman (10) suggested the use of these well-defined chemicals as ovicides.   The mean percentage hatch of aphis eggs obtained from twigs treated with these various compounds are recorded in Table VII, from which it will be seen that the solid hydrocarbons, acenaphthene, anthracene, diphenyl, fluorene and phenanthrene, were used at 0·5%, whereas the liquid hydrocarbons, cymene, dekalin, mesitylene, pseudocumene and tetralin, were tested at 1%.   These low concentrations were used because it was considered that, if one or more of these compounds is responsible for the ovicidal properties of tar oils, this toxicity should be shown at low concentrations.

TABLE VII.

Sprays (All contain 0·6% oleic acid and 0·1% NaOH).	1935. Mean percentage hatch.
Acenaphthene (0·5%) + benzene (2·5%)	$53 \cdot 6 \pm 6 \cdot 91$
Anthracene (0·5%) + benzene (2·5%)	$49 \cdot 8 \pm 4 \cdot 11$
Diphenyl (0·5%) + benzene (2·5%)	$58 \cdot 1 \pm 8 \cdot 90$
Fluorene (0·5%) + benzene (2·5%)	$60 \cdot 8 \pm 4 \cdot 06$
Phenanthrene (0·5%) + benzene (2·5%)	$59 \cdot 9 \pm 3 \cdot 60$
Diphenyl oxide (dibenzofuran) (0·5%) + benzene (2·5%)	$62 \cdot 5 \pm 3 \cdot 72$
Cymene (1·0%)	$64 \cdot 5 \pm 5 \cdot 35$
Dekalin (1·0%)	$53 \cdot 6 \pm 6 \cdot 93$
Mesitylene (1·0%)	$58 \cdot 0 \pm 6 \cdot 09$
Pseudocumene (1·0%)	$64 \cdot 7 \pm 4 \cdot 94$
Pseudocumene (1·0%) + benzene (2·5%)	$68 \cdot 8 \pm 6 \cdot 81$
Tetralin (1·0%)	$67 \cdot 0 \pm 4 \cdot 39$
Tetralin (1·0%) + benzene (2·5%)	$46 \cdot 2 \pm 3 \cdot 84$
Benzene (2·5%)	$70 \cdot 9 \pm 4 \cdot 89$

The results indicate that none of the compounds tested possesses, at the concentration used, any marked toxicity to eggs of *A. pomi*.   The aromatic hydrocarbons which, on the score of solubility in dimethyl sulphate, might be expected to exhibit some degree of toxicity, apparently fail on account of their relatively low boiling point.   The poor ovicidal action of diphenyl oxide to *A. pomi* may be contrasted with its high toxicity to eggs of *C. brumata*, though the concentrations (2·5% and 5·0%) used by Staniland, Tutin and Walton were greater than in the above experiments.

The conclusion to be derived from these trials is that there is no evidence of specificity of ovicidal properties among the fractions and products tested. From the results quoted in Table VI, however, it would seem that slight differences may exist in the ovicidal action of high-boiling neutral oils, soluble in dimethyl sulphate, from diverse sources. Thus, the figure $26 \cdot 6 \pm 5 \cdot 14$, obtained with $6 \cdot 5\%$ P.18 corresponding to $1 \cdot 43\%$ neutral oils soluble in dimethyl sulphate, is significantly inferior to that, $10 \cdot 7 \pm 2 \cdot 32$, obtained with $1 \cdot 5\%$ T.11, containing a lower ($1 \cdot 36\%$) amount of active oils. This difference cannot be explained on the basis of boiling range, for the 50% distillation point of the petroleum oil is $359°$ C., whilst that of the tar oil is $324°$ C. Further, the complete kill obtained with $3 \cdot 0\%$ C.6 ($1 \cdot 6\%$ neutral oil soluble in dimethyl sulphate) suggests that hydrogenation has conferred an enhanced toxicity on the original aromatic hydrocarbons, a feature not revealed by the dimethyl sulphate test. On the other hand, if high-boiling tar oils exist which show ovicidal properties markedly inferior or superior to that represented by the dimethyl sulphate figure, it is probable that such oils would have been recognized in practical use. As no such cases have emerged it may be considered that the dimethyl sulphate test provides an adequate practical method of assessing the toxicity of high-boiling hydrocarbon oils to eggs of *A. pomi*.

SUMMARY.

1. A laboratory method for comparing ovicidal properties, employing eggs of *Aphis pomi* De Geer as test material, is described. By this method, it has been shown that :—

2. Ovicidal properties to *A. pomi* of hydrocarbon oils are determined by their content of high-boiling neutral oils soluble in dimethyl sulphate.

3. There is no evidence of large differences in the ovicidal properties of high-boiling neutral oils soluble in dimethyl sulphate derived from various sources.

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A STUDY OF THE DETERIORATION OF SEAKALE STOCKS, WITH NOTES ON SOME DISEASES OF THAT CROP

By W. BROWN

INTRODUCTORY.

SEAKALE (*Crambe maritima* L.) as a vegetable for forcing is grown in many private gardens but on a market scale its cultivation is to a large extent limited to the neighbourhood of London. Even there the number of growers is relatively few, but the total acreage is substantial and apart from the value of the forced " Kale " as a high-priced vegetable the crop has the merit of requiring a great deal of work during the winter months when work in the fields is at a minimum. The cultivation of seakale thus enables the grower to maintain a larger winter staff than would otherwise be possible.

A short account of the method of cultivation is given in a publication of the Ministry of Agriculture and Fisheries.* In order that the accompanying record of experimental work may be more readily understood, the essential operations, as based on the article cited and on the present writer's observations and experience, will be briefly described.

The crop is propagated by means of root cuttings prepared at the time of forcing, i.e. from November onwards. These cuttings, which are about 3-5 in. long and which should not be thinner than an ordinary lead pencil, are laid in layers out of doors, liberally dusted with lime so as to ward off Club-root disease, and covered with several inches of soil. Beyond a certain amount of additional protection against severe frost, the cuttings require no further attention.

By the middle of April, which is about the usual date for planting out, the cuttings show fine roots (" fibre ") along their length and at one end, while at the other a more or less complete circle of buds arising from a callus ring appears. After a mild winter these buds may be several inches long, when it is convenient to shorten or, in fact, rub off a large proportion of them.

The rooted and budded cuttings are dibbled into the open ground, the upper end being sunk an inch or so below the soil surface. The object of this is to protect the young growth against late ground frosts, but a further advantage will be indicated latter. The standard commercial spacing is 12 in. between the plants in rows 15-18 in. apart. During the summer, in June or better in

* See Sectional Vol. No. 12, " Collected Leaflets on the Cultivation of Vegetables ", 1928, pp. 80-4.

July, the plants should be disbudded, as it is found that, even with plants of best size and quality, the forced product is inferior when more than one " nose " is allowed to develop on the same plant.

In late autumn, when the foliage has either fallen off or is readily detached from the crowns, the plants may be dug up and forced. The thong-like lateral roots are trimmed off a short distance from the main axis, and used for the preparation of cuttings (variously called " thongs " or " jags ") in the way described above, while the plants themselves (called " crowns ") are placed in the forcing pit, the temperature of which should not exceed 60° F. Further details in this connection will be found in the paper referred to above.

The problems relating to seakale cultivation that demand investigation may be grouped under two headings, (1) those relating to the quality of the plants from the point of view of forcing and (2) diseases. Though a number of growers have devoted a good deal of study to this crop, their results are not generally available and, so far as the writer is aware, the information obtainable in the ordinary scientific journals is insignificant in amount. The observations to be described here relate chiefly to the question of forcing quality, but a certain amount of attention was paid to diseases as they presented themselves. Some of these are worthy of further investigation but at the moment enough is known about them to indicate their general significance to the grower of seakale.

The subject of forcing quality is bound up with the question of varietal difference, but it is very difficult to obtain any clear information on the latter point. A few named varieties are listed in catalogues (of which " Lily White " is reputed to be the best) but as things are, each grower's stock is more or less peculiar to himself. It has been grown for many years on his farm and from time to time it has been built up anew, more or less completely, from selected plants. The general experience is that, unless the grower continues to select for propagation purposes, a more or less rapid deterioration sets in and the stock, while still perfectly satisfactory as regards growth in the field, becomes of low value for forcing. A study of the nature of this deterioration formed the main subject of investigation.

EXPERIMENTAL.

The writer's attention was first directed to the problem of seakale deterioration in the early winter of 1930-1 at a farm in Middlesex. A small batch of deteriorated seakale, brought from another farm, was being forced side by side with the owner's stock. The difference in performance was very striking. Whereas the latter showed a fairly even stand and was ready for cutting, the other was nearly all dormant, i.e. as regards the large terminal bud of the crown. On the other hand numerous small side buds, which arise in the axils of foliage leaves as the plants grow in the field, had sprouted giving rise to thin " grassy "

seakale of no commercial value whatever. The deteriorated stock was scrapped, but the writer was able to obtain some 300 cuttings of it which, along with a similar number of the good sort, formed the basis of the material which has since been grown at Slough. By the second year of the work a stock of about 3,000 plants in all had been built up and it has been maintained at that level up to the present time.

Certain growers having suggested that deterioration is brought about by faulty manuring—in particular that over-manuring with nitrogen tends to deterioration whereas potash counteracts this—the work was planned in the first instance as a manurial trial and was continued as such for four years. The two stocks, which will be referred to throughout as " good "* and " bad ", were grown side by side on plots which were differently manured, and their performance recorded each year. Meanwhile, propagation from individual plants of the good stock was begun. The original line of experiment was abandoned after four seasons when it was seen that manurial effects were not as important as was originally supposed, and while small lots of the good and bad stocks are still being carried on, most of the field space is now given over to some forty clone populations which have been developed. A variety of subsidiary experiments has also been carried out. The work may therefore be conveniently described under the following headings :—

(1) Manurial treatment in relation to deterioration.

(2) Behaviour of single-plant selections.

(3) Miscellaneous experiments.

(4) Diseases.

(1) MANURIAL TREATMENT IN RELATION TO DETERIORATION.

A series of fourteen plots (20′ × 10′ each), cut out of old grassland, were available and the following manurial scheme was adopted, the figures given representing rates per acre :—

2 plots, no artificials.

2 ,, ,, ,, + 2 tons lime (slaked).

2 ,, 6 cwt. potassium sulphate.

2 ,, ,, ,, ,, + 2 tons lime.

2 ,, 4 cwt. ammonium sulphate.

2 ,, ,, ,, ,, + 2 tons lime.

1 plot, 10 cwt. superphosphate.

1 ,, ,, ,, + 2 tons lime.

* As will appear later, the good stock was not all good, even in the early years of the experiment, and has now almost completely deteriorated.

The dressing of nitrogen was applied in each year, usually in two doses, in May and June, whereas the other artificials were worked into the top soil just before planting. The lime was added during the winter when the ground was being trenched.

As the soil was a light loam which had carried a poor sward of grass, all the plots received a dressing of farmyard manure at the rate of approximately thirty tons per acre which was worked into the subsoil. The same treatment was given in the 3rd but omitted in the 2nd and 4th years. Manurial treatment was thus fairly liberal and resulted in stronger growth of the plants than is commonly seen on farms.

Even in the first year of the experiment Club-root disease, which must be ascribed to contamination of the farmyard manure used, made its appearance on plants in the unlimed plots and in the second year was much worse. It was necessary therefore to lime the unlimed plots. In the winter of 1932-3 each of the latter was limed twice at the rate given above, and in the following winter the treatments were repeated, so that by the fourth year all the plots had received the same amounts of lime. By this means the Club-root trouble was reduced to small dimensions. In the light of experience it was unfortunate that the same liming treatment had not been adopted for all the plots, but in fact no difference arising from the irregularity in liming was noted. In substance, therefore, the experiment resolved itself into a comparison in quadruplicate plots (duplicate with phosphate) of the effects of excess potash, nitrogen, or phosphate on forcing quality.

Apart from the first year, in which the stock was insufficient for the purpose, fifty-four plants of the good stock and fifty-four of the bad were grown on each plot. The thongs of the two sorts from each of the various plots were kept separate and in each succeeding year were replanted on the same plot. At the end of the fourth season, therefore, there were plants of the good and bad stocks which had been grown for four years in presence of a distinct excess of potash, nitrogen or phosphorus, and others for which the manurial treatment had been more balanced. In each year the crop was forced, as a rule in 3-4 relays of batches over the period November to February, and a full record was made of average crown and kale weights and of the kale quality of the various sorts.

Before proceeding to present these results in summarized form it will be convenient at this point to outline the characteristic features of good and bad types of seakale, as seen in the forcing pit. The distinctions were more clearly seen at a later stage of the work when clones of good and bad quality were available for comparison. They are as follows :—

The good type is characterized by its stoutness, individual leaves being wide in cross section especially towards the base ; the habit is compact, so that

material of this type lends itself readily to packing without damage ; the small side buds which lie below the large terminal bud remain dormant. Conversely, the bad type may be described as spindly and sprawly, with a tendency for side buds to develop and produce " grassy " worthless seakale. The colour of the bad type is pinkish, as against the creamy or slightly yellow of the good type. For market requirements, the leaf laminae should be as small as possible, and in this respect also the stout compact type is superior to the other. Under comparable conditions, there is no material difference in the average amount of seakale produced by crowns of the two types.

The features outlined above are somewhat modified by a number of factors of which the most important are the width of the crown, the temperature of the forcing pit and the time of year at which forcing is carried out.

Width of crown is important to the extent that the total weight and the stoutness of the kale increases with crown width, but only up to a point. Very large crowns give no proportionate increase in weight of kale and very often give no increase at all. It is possible that this rule, which is in agreement with growers' experience, may be modified by suitable manurial treatment, but speaking generally it may be said that a crown width of 1-1¼ in. represents the limit beyond which any further increase in size gives no appreciable increase in yield or quality. On the other hand, crowns of small width, even when of good intrinsic quality, give kale which is lacking in stoutness and is of reduced weight. Increase in width of the crown accentuates any tendency to sprawliness and to the sprouting of lateral buds. Hence the distinctions between good and bad types tend to disappear when the crowns are small.

The temperature of the forcing pit affects both the speed of forcing and the quality of the product. With higher temperatures the kale is less stout and there is some evidence that the amount of lamina development is increased.

The effect of time of year is, however, the most notable. In early forcings, beginning say at the end of October, the difference between good and bad types is pronounced. At this period the terminal buds of the latter are liable to remain dormant while the small lateral buds develop freely and produce " grass ". Crowns of good type force at this stage more quickly and evenly, but even these are not entirely satisfactory. Thus, while in later forcings a crown of good type would show 5-8 leaf stalks extended, a similar crown when forced early in November might have only one or two stalks of full length, with perhaps a few short ones in the centre of the bud. As a result of this the weight of kale per crown is low in early forcings, even when extra time is allowed, and the habit moreover is inferior, as the long outer stalks tend to diverge and often to twist.

In December forcings both types start fairly evenly, and later still, e.g. at the end of January, the bad type forces somewhat more quickly than the good. The sprouting of side buds which is characteristic of the bad type is still

pronounced in December forcings, but towards the end of the season is much reduced. With both types the flowering axis tends to "push" as the season progresses, and this tendency is greater in the good than in the bad type.

Representative samples of bad and good types are illustrated in Figs. 1 and 2.

The outstanding result of the manurial experiment was that no treatment made the bad stock good and that the good stock remained more or less good under all treatments. A few good plants were present in the bad stock up to the second year but after that they disappeared entirely. The good stock has also deteriorated as is shown in Table I, which gives the percentage of crowns producing stout seakale in the grouped series of manurial plots. Each figure in the Table is the average obtained from three forcings, the component figures showing good agreement. Comparable data are lacking for the first year of the experiment (1931), as facilities for forcing were not then available at Slough.

TABLE I.

Percentage of crowns giving stout seakale under different conditions of manuring.

	1932.	1933.	1934.
Control Plots	48	37	20
Potash Plots	54	54	41
Nitrogen Plots	42	43	34
Phosphate Plots	40	38	24

As has been stated, the basis on which the plants were graded was stoutness of leaf-stalk. It is admitted that the standard adopted might vary somewhat in different years. Furthermore, the average crown weight decreased to some extent in each succeeding year (3·05 oz. in 1932, 2·8 in 1933, 2·5 in 1934) and this in itself would rather reduce the percentage of plants which gave stout seakale. There is, however, no doubt that deterioration has taken place. The figures in the Table are underestimates, since all small crowns, even those of good type, would be graded as producing inferior seakale. The percentage of plants of good forcing quality was undoubtedly higher, and probably much higher, than 50% in 1932, whereas in the past year (1936), it had definitely sunk to 17%, as determined by foliage examination of the growing crop. It was unfortunate that this mode of distinguishing between good and bad plants, which was not discovered until 1935, i.e. after the manurial experiment was finished, was not known earlier, since by that means the progress of deterioration could have been more accurately followed.

The criticisms in the foregoing paragraph do not apply to gradings made on any one date, and therefore there is a definite suggestion that manurial

treatment affects the rate at which deterioration proceeds—viz. that it is greatest in the control plants and in those treated with phosphates, least in the potash and intermediate in the nitrogen plots. To the extent indicated therefore, the system of manuring influences the progress of deterioration, but it is doubtful if much importance is to be attached to this result. In the light of what will be pointed out later regarding the rooting habits of good and bad forcing types of plants, it is probable that the general level of manuring and the depth to which the soil is worked are factors which would have to be taken into account in this connection.

Throughout the run of the manurial experiment a complete record was kept of the weight of crowns and of forced seakale from both stocks on all the plots. No substantial difference arising from the different inorganic manures added was established. Perhaps the liberal treatment with farmyard manure was sufficient to mask any effects of the artificials in this respect.

The growth of the two stocks on the variously manured plots was followed in detail in season 1933 by recording on two dates the sizes of the above-ground portions of the plants. Four grades of size—large, medium large, medium and small—were decided upon and the whole crop of some 1,500 plants in the manurial plots was thus graded. In thirteen of the plots the bad stock was clearly ahead of the good in general vigour of growth ; in one plot only was there no noticeable difference. The results of this survey, summarized for the whole fourteen plots, are set out in Table II, which gives the percentage of plants which fall into each size-class.

TABLE II.

Percentage of plants, in good and bad stocks, arranged according to grade at two periods.

Date.	Stock.	Large.	Medium large.	Medium.	Small.
June 19th	Good.	28·3	37·3	24·4	10·0
	Bad.	46·8	35·8	13·2	4·2
July 12th	Good.	26·4	39·4	27·4	6·8
	Bad.	51·4	32·8	12·9	2·8

The figures in the Table show clearly that the bad stock was the more vigorous in growth on both dates of inspection.

In a smaller experiment, with 250 plants of each stock, the growth vigour of the plants on July 12th was correlated with the diameter of the crowns when lifted at the end of October. In Table III the first column of figures gives the number of plants (out of 250) which fell into the various size-classes in July, and the remaining columns give the number of crowns of various sizes at lifting time.

TABLE III.

Correlation between vigour of growth in summer and crown diameter at time of forcing.

Stock.	Size-Class.	No.	Missing.	¾ in. or less.	⅞ in.	1 in.	1⅛ in.	1¼ in.	1⅜ in.	1½ in. or more.
Good	Large.	51	0	0	9	21	12	6	2	1
	Medium large.	96	0	11	36	27	20	1	1	0
	Medium.	84	4	49	24	6	1	0	0	0
	Small	19	6	12	0	1	0	0	0	0
Bad.	Large.	135	0	4	13	32	42	21	14	9
	Medium large.	80	0	13	21	23	12	7	3	1
	Medium	26	2	13	5	3	1	2	0	0
	Small.	9	3	3	0	0	1	0	1	1

This Table again substantiates that the bad stock is characterized by greater growth vigour in the field and that in both stocks the groups which are graded as vigorous in July tend to give the largest crowns in October. These results were borne out by the greater average crown-weight of the bad stocks at the time of lifting, viz. :—

Bad stock, 3·32 oz. ; good stock, 2·86 oz.

In carrying on the stocks from year to year a record was kept of the number of root cuttings ("thongs") made. A definite standard of size was adopted, viz. a length of 4 inches and a diameter of at least ¼ inch (comparable to an ordinary lead pencil). With the good stock it was necessary in the first year to break this rule, i.e. in certain cases to cut thinner thongs, in order that a sufficient number of cuttings should be obtained. Even so, the figures of Table IV illustrate the invariable experience that the root system of the bad stock was more satisfactory for the preparation of standard cuttings. The figures in the Table give the average number of cuttings per plant.

TABLE IV

Relative number of thongs from good and bad stocks over a period of four seasons.

Year.	Good Stock.	Bad Stock.
1931	2·8	4·2
1932	4·6	4·8
1933	2·3	3·8
1934	2·5	4·0

Thus, in any mixture of good and bad types in a stock there is the likelihood that a higher percentage of thongs will be cut from the bad type than

corresponds with the original admixture, so that in successive years the percentage of the bad type will tend to increase. This fact alone, in the writer's opinion, is the chief if not the only reason for the observed deterioration of a stock.

The difference in rooting habit indicated above is shown in the early behaviour of root cuttings. Thongs of the bad forcing type begin to " fibre " more quickly than those of good type under the same conditions.

A third point of difference between the two stocks, as seen in the field, shows up clearly in the autumn, e.g. in October, and especially after there has been a slight ground frost. This difference is still more clearly marked with the clone selections. At this date the leaves of the good type have practically all collapsed and fallen to the ground whereas in the other they are still vigorous and attached to the plant. In other words the good type of plant ripens first. This difference throws a light on the experience in the forcing pit where it is found that early forcings of the bad stock show a pronounced tendency to dormancy of the main buds.

(2) BEHAVIOUR OF SINGLE-PLANT SELECTIONS.

In the spring of 1932, rooted and callused thongs were taken at random from the good stock and planted separately in good garden soil. Though all these were not carried on, the first year's records showed that 26 out of 34 were of good forcing type. During the winter of 1933-4 a further batch of selections was begun, but in a somewhat different manner. A number of plants of good size were marked out in the field. When these were lifted only the ones which had good root systems, i.e. which would give about six thongs for propagation, were used. The thongs were put to callus in separate beds and the crowns were forced in the usual way. Of this batch it is interesting to note that only 8 out of 29 proved to be of good forcing type. At that time the proportion of good plants in the stock was undoubtedly much higher. The method of selection, viz. the choice of good sized plants with good root systems, was clearly responsible for the large proportion of bad plants obtained. This result is a further confirmation of what has been said above regarding the rooting habits of the two types of plants.

Many of the bad types were discarded at once, and some after a few years, but altogether 38 selections are now being carried on. Of these 24 are are of good and 14 of bad type. Of some the records of performance are available over five, of others over four years.

The result up to the moment is that no definite change has been noted in the forcing character of any of the selections. From time to time a suspicion of deterioration in some of the good types has been recorded, but so far it has

not been confirmed in the year following.   There is from year to year a certain amount of fluctuation, but this can be set down to variations in the dates of forcing.   The good types are not all the same, though many are indistinguishable and some are definitely inferior to others.   A short description of a few of the selections of good type will serve as illustration.

Selection A : Over five seasons this has invariably given stout seakale of compact habit ; lateral buds almost always remain dormant in the forcing pit.

Selection B : A type characterized by weak, bluish foliage which dies down very early ; the root system is relatively good ; crowns force very quickly at the beginning of the season ; the seakale is not stout enough but the habit is compact and side buds remain dormant.

Selection N : A type which produces stout, compact seakale but shows a distinct tendency for a number of side buds to develop.

Selection U : Later in maturing than B and forces more slowly early in the season ; root system weak and finely divided ; excels B in stoutness of seakale produced.

Only in one case—Selection E—which is a good though weakly growing type, has anything untoward occurred.   In 1936 six plants of definitely bad type suddenly appeared in a stock of about 150 plants.   As this is the only example of deterioration which has been met with so far, the most plausible explanation at the moment is that accidental admixture is responsible.

With a few of the original selections, considerable difficulty has been encountered in maintaining the stock, and in fact some have had to be abandoned.   The plants were small with poor root systems, and satisfactory thongs could hardly be obtained.   Possibly fungoid disease was the cause of this, but the question was not taken up.
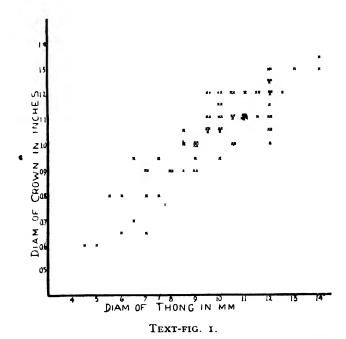
The results so far obtained with these clones have not confirmed the assertions of growers that good plants tend to revert.   The occasional throwing of sports of bad type by good plants is not impossible, but with one doubtful exception this has not happened yet in the history of these clones.   While the results already obtained explain why, in a mixture of good and bad types, the method of propagation tends to increase the proportion of the latter, they have not as yet indicated the manner in which the bad type arises in the first instance.

During the summer of 1935, by which time plots of the clones were available for comparison, a distinct difference was noted between the leaves of good and bad types.   Later it was learned that the fact was known to some growers. The difference lies in the serrations of the leaf margins.   These serrations are numerous and sharply pointed in leaves of bad type (termed " hollyleaved " by growers) ; in those of good type they are larger and blunter or even lobe-

like. The difference is not clearly seen in young plants ; it is best seen when the plants are in full growth, and then it is equally noticeable in the full-grown leaves and in the young crumpled leaves towards the centre of the plant. Some of the clones, viz. the weakly growing type with bluish leaves, show the sparsely notched flat-lobed appearance most distinctly.

(3) MISCELLANEOUS EXPERIMENTS AND OBSERVATIONS.

(a) *Relation of thong-size to crown-size.* While the length of the thong is, within wide limits, of no importance, the width has a direct effect upon the diameter of the resulting crown and therefore, within limits, on the stoutness of the kale produced.

TEXT-FIG. I.

Illustrating relation between diameter of thong and diameter of the resultant crown.

Text-fig. I gives the result of an experiment in which eighty thongs of known diameter were followed through a season's growth and the diameters of the resultant crowns determined. While there is obviously a good deal of irregularity, the general tendency is clear—that the thong of a greater diameter is the more likely one to produce a crown of large diameter. Some idea of the minimum thong necessary to produce the commercially ideal crown can be obtained from such a diagram, but variations in soil and season would also have to be taken into consideration.

(b) *Natural disbudding of plants.* The growers' practice is to disbud the plants at least once during the growing season so as to avoid the occurrence

of multiple-nosed crowns. In a number of experiments with plants of the good and bad stocks, disbudding was purposely omitted and records were obtained illustrating the natural disappearance of buds as the season progressed. At the time of planting in the field the rooted cutting may have a more or less complete ring of buds at its upper end. The following records of two plants will serve to show how this large number of buds is progressively reduced by a natural process.

(1) Cutting with a complete ring of buds, planted on April 16th ; on June 10th, the plant showed 3 large and 3 small buds ; on July 2nd, 3 large and 1 small bud ; the crown when lifted in winter had 1 main bud and 2 secondaries (corresponding to the three large buds of July 2nd).

(2) Cutting with a complete ring of buds, planted on April 16th ; on June 10th, the plant showed 1 large, 2 medium and numerous small buds ; on July 2nd, 1 large and 1 small bud ; on lifting the crown had 1 bud only.

Observations of this nature showed that, in general, the final condition of the plants as regards buds could be foretold accurately from an inspection in July.

In records obtained over three years, plants of the good stock have shown a distinctly greater tendency than those of bad stock to disbud themselves. The data obtained, in terms of percentage, are given in Table V.

TABLE V.

Relative amounts of natural disbudding of good and bad stocks.

	Stock.	1-nosed.	2-nosed.	3- or more nosed.
1932 ..	{ Good.	38	52	10
	{ Bad.	30	42	28
1933 ..	{ Good.	48	38	14
	{ Bad	35	40	25
1934 ..	{ Good.	56	22	22
	{ Bad.	20	44	36

The results in Table V and the comparative forcing behaviour of good and bad types can be summarized in the statement that the former show greater dominance of the apical bud. In the field, with no artificial disbudding, the leading bud in plants of good type has a greater capacity to suppress smaller buds, and this feature is more pronounced in the forcing pit. Whereas the apical bud of a plant of good type is dominant to side buds at any period of the forcing season, a progression with plants of bad type can be noted. Early

in the season, e.g. in November, side buds are liable to be dominant to and to suppress the main bud ; later, as in December, both main bud and side buds force freely ; but towards the end of the season the main bud tends to become more dominant.

(c) *Polarity of roots*. As is well known, the formation of buds at the morphologically upper end and of new roots at the other end of a root cutting is independent of the orientation of the latter in the ground. On the other hand, the development of buds at the wrong end of roots has frequently been noted on the stubs of roots which are left on the crowns while the latter are being forced. It is very common to find buds arising among the roots under these conditions, but the majority of these are lateral to the roots, generally at wounds. Nevertheless numerous instances have been noted in which their origin is strictly terminal, viz. on the cut surface. It is suggested that this reversal of polarity may be due to temperature or moisture gradients within the soil of the forcing pit. Crowns with such abnormally placed buds have been planted, but these buds have invariably died off and the crown has formed a callus and buds in the normal position.

(4) DISEASES.

CLUB-ROOT. The occurrence of this has already been mentioned. Heavy liming prevents it from becoming serious, though in 1936 it was unpleasantly prominent in plots which had been dunged and which had been limed at the rate of two tons per acre in the preceding year. Affected roots are swollen and soon become black. No obvious effect on plant growth has been observed and when the galls are confined to the side roots the only damage is the loss of the latter for the preparation of cuttings. Very often, however, galling of the base of the crown occurs and then the loss is serious, as such a crown rots from below upwards when put into the forcing pit. Forcing of the bud is thereby retarded and often completely prevented.

COPPER-WEB DISEASE due to *Rhizoctonia Crocorum* has been met with occasionally, more particularly on the distal parts of roots. Very rarely has its presence interfered with forcing of the crown.

DISEASE DUE TO *Rhizoctonia Solani*. In the forcing pit the leaf stalks, usually at the base but sometimes higher up, are blackened and rotted. Exceptionally the whole bud is attacked so that the seakale topples over. From such affected plants *Rhizoctonia Solani* has regularly been isolated, and inoculations of this fungus into the developing kale have proved successful. This trouble is probably best controlled by seeing to it that the tops of the crowns are raised above the level of the soil of the forcing pit.

A more interesting occurrence of this fungus is seen on the plants in the field. It has been noted above that a considerable amount of natural disbudding takes place as the plants grow. Some buds forge ahead of the others and the latter in due course are suppressed. As these weak buds are in process of dying they show a blackened cortex, and from this *Rhizoctonia Solani* is readily isolated. From time to time the fungus attacks the leading buds also and so the whole plant is killed. Hence the bare patches which have occasionally occurred. Usually, however, the fungus is able to destroy the weakened side buds only, so that up to a point its activity is beneficial to the cultivation of the crop.

A number of subsidiary experiments, in which cuttings were planted so that their tops were just above ground level, tend to confirm the view that the fungus plays a significant part in bringing about natural disbudding. Such high planting would serve to remove the developing buds from the influence of soil fungi. Table VI gives the results of one such experiment in which a comparison was made of the final condition of crowns derived from high-planted as against normally planted thongs. Two other experiments in different years gave the same result.

TABLE VI.

Effect of method of planting on natural disbudding.

Stock.	Method of Planting	Condition of Crown			
		1-nosed.	2-nosed.	3-nosed.	4- or more nosed.
Good.	{ High.	3	5	9	5
	{ Normal.	13	5	3	2
Bad.	{ High.	1	8	4	10
	{ Normal	5	11	6	3

BLACK STREAKS IN ROOTS. Not infrequently one sees black dots in cross sections of roots. These correspond to strands of blackened tissue, usually but not always fairly central, which run for some distance along the root. It was early seen that forcing quality is not closely related to the presence or absence of black streaks in the roots inasmuch as they were as frequent in the good stock as in the bad. The writer's practice has been to discard for purposes of cuttings any roots in which the internal blackening is pronounced. In one experiment a number of internally blackened thongs were found to form buds and roots in a perfectly satisfactory manner. Twenty-seven such thongs were planted out and though the growth was uneven, all except one gave crowns of normal average size in the autumn. Their forcing behaviour was however very bad, but this result was due, in part at least, to Club-root. Thongs cut

from these plants showed no more internal blackening than usual. Further experiments as to the cause of the blackening and as to its significance in the production of seakale are in progress.

SOFT-ROTTING of the crown, with the production of a grey coloured semi-liquid mass, has occasionally been observed.

BAD FIBERING of the crowns in the forcing pit appears to be due, apart from some of the fungi mentioned above, to too high a temperature in the forcing pit. This is most often met with in crowns which are situated immediately above the water pipes. The cut ends of the roots become black and no new fibres are produced. With high moisture content of the soil the ends of the roots become rotted. The effect on the kale is a slowing down of forcing.

VIRUS DISEASE. The sporadic occurrence of leaf mottling has been observed and is suggestive of virus disease though it has not been proved to be such. The appearance usually takes the form of a vein clearing, the leaf tissue lying along the veins being of a lighter green than the rest of the leaf. This effect is much more distinct in some plants than in others. There is an indication that the clones which are the weakest growers are the ones which are most affected.

The possibility that deterioration in forcing quality might be due to virus disease was considered and for this purpose a number of grafts were made. Altogether twenty-six such composite thongs were made, the upper portion being of good type and the lower of bad. All these graftings were successful and the plants grew in the usual manner. When these crowns were forced they all gave kale of good type. Thus, in spite of organic union, no bad feature had been communicated to the apical bud. The experiment was continued further by cutting off the good upper part of the crown, in those cases where the junction was still visible, rooting it and growing on for another season. Again the kale produced was of good quality and the thongs produced gave plants of good type. There was clearly no evidence of a transmitted virus dsease.

DISCUSSION.

The foregoing results indicate clearly that the main cause of the deterioration of seakale stocks is the presence of plants of bad type, the proportion of which tends to increase on account of the method of propagation adopted. Plants of bad type are strong growers, and what is probably more significant, begin to grow more quickly and to form a larger root system in the spring. As the commercial spacing in the field is rather close, the roots are under highly competitive conditions so that the intrinsic difference in root-growth is probably accentuated where the two types are grown intermingled. This would tend to

the more rapid submergence of the good type in the stock. That conditions are highly competitive is indicated by the fact that plants at the edges of the plots have as a rule been found to possess very long roots.
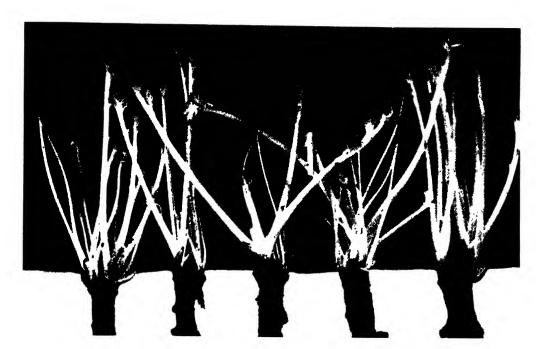
While manurial practices, depth of cultivation, spacing, and seasonal effects are factors which would appear likely to influence the rate at which deterioration proceeded, the major fact is that deterioration will continue so long as plants of bad type are present. Hence the desirability of their removal. In a stock which contains a small proportion of such plants, rogueing could be carried out in the field on the basis of leaf characters, at a time when the root system is small. Rogueing at a later date would be less effective, inasmuch as broken roots of a size suitable for thongs would be left in the ground and might be used for cuttings. Where there is a high percentage of bad plants in the stock, the only feasible procedure is to build up the stock anew from plants of good forcing quality. Merely to propagate from some good plants each year and in due course to add the new stock to the old so as to give a general leavening effect would seem to be a waste of effort. The stocks, old and new, should be strictly segregated, until such time as the old one can be discarded entirely.

A certain amount of deterioration in relation to cropping capacity may also be traceable to fungoid agency and to virus disease. Further work is necessary to determine these points.

The writer's experience of the behaviour of single-plant selections runs over five years only and is admittedly limited. The possibility that plants of good sort may throw sports of bad type from time to time cannot be overlooked ; indeed, occasional plants have been observed which show peculiar features, e.g. red or albino leaves or portions of leaves, and these have presumably arisen by sporting. Nevertheless the fact remains that no clear deterioration has arisen in the twenty-four single-plant selections of good type which are being maintained, and it will be noticed that the system of cultivation was such as to lead to a rather rapid deterioration of the originally good—and mixed—commercial stock. Further experience of the behaviour of these selections will show how far a liability to sporting will interfere with the maintenance of a pure stock of good type.

The problem of the effect of manuring on the forcing behaviour of a good stock has not been tackled in this work. The influence of the standard manurial constituents—K, N and P—on the maturing of the crop and on the finer qualities of the forced product would be worthy of careful study, but it did not seem advisable to consider such points until a pure stock of plants of good type was forthcoming.

FIG. 1
Crowns of forced seakale of bad type

FIG. 2.
Crowns of forced seakale of good type.

large amount of detailed recording which he carried out in season 1933, and for much help in tabulating the notes relating to several seasons' work.

SUMMARY.

1.   In a study of the deterioration of a good stock of seakale, manurial treatment was found to be a factor of secondary importance. The cause of deterioration is the presence in the good stock of a number of plants of bad forcing type, and the proportion of these tends to increase as a result of the method of propagation adopted.

2.   The characteristic differences between plants of good and bad forcing type are outlined. These differences relate to growth vigour in the field, leaf shape, date of maturity, development of root system and forcing behaviour at different periods of the season. Plants of good type show, both in the field and in the forcing pit, a greater tendency for one bud to become dominant over all others.

3.   The history of a number of clones, some of good and some of bad type, is recorded. None of the clones of good type has, up to the present time (i.e. after 4-5 seasons) shown any significant deterioration.

4.   Diseases of seakale, as they have been encountered in the experiments, are recorded and briefly described.

INCOMPATIBILITY AND STERILITY IN THE SWEET CHERRY, *PRUNUS AVIUM* L.

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INTRODUCTION.

THE work on incompatibility and sterility in cherries and other fruits, in progress at the John Innes Horticultural Institution, has been reported upon from time to time (Crane 1923-7, Crane and Lawrence 1929-31). The experiments have been continued each succeeding year on trees grown in pots, under the methods of control described in earlier papers. The last report, published six years ago, dealt with the genetical aspects of the investigations and in particular with the hereditary basis of incompatibility. In the present report the results obtained during the last six years are described, and both the practical and the genetical aspects of the experiments are considered. The results of the work with seedlings are summarized, but those obtained from pollinations with established varieties of cherries are given in detail.

The material used in the experiments here reported upon has consisted mainly of varieties and seedlings of *Prunus avium,* the Sweet Cherry. But *Prunus Cerasus*, the Sour Cherry, and the hybrids between these species, collectively known as the Duke Cherries, have been used in the pollinations, and to a small extent *Prunus decumana* and *P. cantabrigiensis*.

In the genus Prunus the basic chromosome number is 8. At the time the last report was published a number of the varieties of Sweet Cherry we had used were thought to be aneuploids possessing one or more chromosomes beyond the diploid number (Darlington 1928-30). Since then Darlington (1934) has re-examined a number of such varieties and found them all to have 16 chromosomes ; and there seems little doubt that all varieties of the Sweet Cherry are diploids (2x = 16). This is in agreement with the cytological studies of Ewert (1922), Kobel (1927) and Prywer (1936). Of the other species used in the experiments, *Prunus decumana* is diploid, 2x = 16 (Darlington 1928) ; the Sour Cherries, *Prunus Cerasus*, and the Duke cherries, *P. Cerasus* x *P. avium*, are tetraploid, 4x = 32 (Kobel 1927, Darlington 1928). *Prunus cantabrigiensis* is also tetraploid, 4x = 32 (Fabergé, unpublished). This species was described by Stapf (1926). It is a native of China and was formerly confused with *Prunus pseudocerasus*. In this country it has been distributed under the varietal name of " Chinese Early ".

The varieties used in the experiments and the results obtained from numerous self- and cross-pollinations are given in the appended Table IX. In

this table the varieties are arranged in alphabetical order. Cross-incompatible pollinations, when they occur, are detailed at the beginning of the columns and are printed in italics. Then follow the results obtained from inter-varietal compatible pollinations, and finally, at the ends of the columns, the results are given of pollinations between the diploid Sweet Cherries and the tetraploid Sour and Duke Cherries.* Hitherto but few of the results have been published and practically none since 1925. Prior to 1925 the pollinations were made on a comparatively small scale ; since then a large number have been repeated, and the total results are given in Table IX. The numbers recorded in the Tables are of " fruits matured " not fruits set.

INCOMPATIBILITY.

The results of the pollinations between the varieties of the Sweet Cherry detailed in Table IX show that all the varieties tested are self-incompatible and that cross-incompatibility is common. As a rarity, fruits have formed following both self- and cross-incompatible pollinations. The proportion of such fruits, 0·1%, is, however, very small and their occurrence is discussed later. Out of a total of 66 varieties investigated 45 have been found to belong to 11 incompatible groups. A few of these varieties have only recently been assigned to their respective groups, and in some cases the pollinations require repetition ; but from general experience we have little doubt of the validity of the available results. The remaining 21 varieties are, so far, independent of any group, but some of them have not been extensively used, and further work is necessary before it can be concluded that they do not belong to any of the 11 groups so far established, or to additional groups.†

Self- and cross-incompatibility are often referred to as self- and cross-sterility. It may be pointed out, however, that when dealing with the phenomenon of incompatibility we are not concerned with sterility in the strict sense of the word, as in the former the pollen and the ovules, or at least a high proportion of them, are functional. The failure to obtain fruit from self- and cross-incompatible pollinations is due to the arrest of pollen-tube growth in the stylar tissue. The pollen tubes fail to reach the ovary and fertilization cannot take place ; and, since the formation and development of the fruit is dependent upon fertilization, the young unfertilized fruits cease to grow, and fall from the trees at an early stage (see Fig. 1). In the compatible pollinations,

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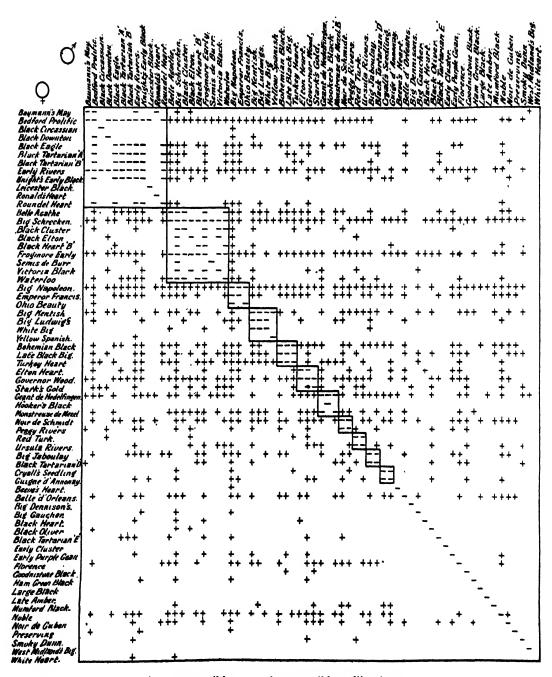
* In Table IX. the tetraploid varieties are indicated by an asterisk.

† While this paper has been in the press the results of another season's work (1937) have become available and indicate that Late Amber and West Midlands Big. belong to Group IV. The results are :—

        Late Amber × Big. Kentish, 24 flowers, 0 fruit ;
        Big. Kentish × Late Amber, 17 flowers, 0 fruit ;
        West Midlands Big. × Big. Kentish, 59 flowers, 0 fruit.

TABLE I.

Incompatibility in varieties of the Sweet Cherry.

Row labels (♀, top to bottom):
Baumann's May, Bedford Prolific, Black Circassian, Black Downton, Black Eagle, Black Tartarian 'A', Black Tartarian 'B', Early Rivers, Knight's Early Black, Leicester Black, Ronald's Heart, Roundel Heart, Belle Agathe, Big Schrecken, Black Cluster, Black Elton, Black Heart 'B', Frogmore Early, Semis de Burr, Victoria Black, Waterloo, Big Napoleon, Emperor Francis, Ohio Beauty, Big Kentish, Big Ludwig's, White Big, Yellow Spanish, Bohemian Black, Late Black Big, Turkey Heart, Elton Heart, Governor Wood, Stark's Gold, Géant de Hedelfingen, Hooker's Black, Monstreuse de Mezel, Noir de Schmidt, Peggy Rivers, Red Turk, Ursula Rivers, Big Jaboulay, Black Tartarian 'D', Cryall's Seedling, Guigne d'Annonay, Beeve's Heart, Belle d'Orléans, Big Dennison's, Big Gaucher, Black Heart, Black Oliver, Black Tartarian 'E', Early Cluster, Early Purple Gean, Florence, Goodnestone Black, Ham Green Black, Large Black, Late Amber, Mumford Black, Noble, Noir de Guben, Preserving, Smoky Dunn, West Midland's Big, White Heart.

(+ = compatible ;   — = incompatible pollinations.)

however, although the same pollen and ovules (in different combinations) may take part, the pollen tubes travel the full length of the style, the male and female nuclei fuse and the fruit develops.

The co-incompatible varieties, the groups to which they belong and the effective and non-effective pollinations are shown in Table I.

The gross totals of (a) self-pollinations, (b) cross-incompatible pollinations and (c) cross-compatible pollinatons made between varieties of the Sweet Cherry and the results obtained are summarized in Table II. The results obtained from pollinating the Sweet Cherry with the Sour, Duke and other species are also given in this Table.

TABLE II.

Sweet Cherries (*P avium*).	No of flowers pollinated.	No of fruits matured	Percentage matured.
Self-pollinations	49,160	30	0·06
Cross-incompatible pollinations	30,847	33	0·11
Cross-compatible pollinations	130,356	29,439	22·58
Pollinated by Sour varieties (*P Cerasus*) ..	4,205	841	20·0
Pollinated by Duke varieties (*P Cerasus / P avium*)	4,947	747	15 1
Pollinated by *P cantabrigiensis*	101	3	2·9
Pollinated by *P decumana*	387	101	26·1

The experiments of many other investigators, amongst whom may be mentioned Gardner (1913), Schuster (1922-5), Florin (1923), Wellington (1926), Tufts, Henrickson and Philp (1926), Sachoff (1931), Schandert (1932), Einset (1932), Kobel and Steinegger (1933), Wenholz (1936) and Johansson and Calmar (1936), have shown that in the Sweet Cherry self-incompatibility is the rule and that it is exceptional to obtain a fruit from self-pollination. Several of the above investigators have also found examples of cross-incompatibility, and in their recent paper Johansson and Calmar (1936) have shown that the varieties Coe's Transparente and Lucie are co-incompatible and belong to the same group as Early Rivers, i.e. our Group I.

References to the occurrence of self-compatibility in the Sweet Cherry are rare, and lack conviction. Pashkewitch (1930) reports that at one Russian station the varieties Yellow Donissen, Big. Napoleon and Black Dayber are self-fertile under natural self-pollination, whatever that may be, and set 44, 57 and 64% of fruit respectively (see Russian text, pp. 38-9). In our experiments Big. Napoleon set only 2 fruits out of a total of 1,429 flowers self-pollinated. This variety has also been extensively investigated in other countries and has been found to be almost or entirely self-incompatible. The results reported by Pashkewitch are therefore exceptional, but the statement he makes that the results were obtained under natural conditions suggests that the

pollinations were not controlled, and if such was the case the results are of no value. In other stations in Russia the three varieties mentioned above, and many others, were found to be self-incompatible. The short summary in English appended to the report definitely states that the varieties are self-fertile, and this has been quoted in English publications. This summary, however, appears to be misleading, and we are indebted to our colleague Dr. A. C. Fabergé for translating the Russian text of Pashkewitch's report.

In a recent statement Salisbury (1935) implies that the variety Hertfordshire Black is self-compatible, but no details are given. Apparently Hertfordshire Black is one of the varieties in cultivation, allied to, or which come under the name of, Corone or Caroon. This appears to be the name of a group, consisting of a number of similar individuals, and not the name of a single variety. Referring to Corone, Bunyard (1925) for example, states, " This comes fairly true from seed and there are therefore many varieties in cultivation which exhibit slight differences." It therefore seems possible that in practice very similar but distinct individuals may be cultivated under the name of Hertfordshire Black, and that they may effectively pollinate each other. We have endeavoured to obtain this variety, but so far without success, and we should be grateful for any information as to where either young trees, or shoots for budding and grafting, of Hertfordshire Black can be obtained. A self-compatible variety would be of outstanding value in genetical research, and since theoretical conclusions indicate that a self-compatible variety is likely to be universally effective on self-incompatibles it might be of considerable practical value.

STERILITY.

It is shown in Table II that the average set of fruit obtained from the cross-pollinations made in these experiments is 22%. This approximates to a good yield, but as the results detailed in Table IX show, among these compatible crosses very considerable variation occurs. Environmental factors such as the age of the tree, the kind of crop it carried in the previous year, and whether flowers were borne abundantly or otherwise, are frequent causes of such differences. Experience has shown that the results of individual pollinations cannot always be relied upon, as in many cases an initial poor set has on repetition given a high yield. In particular, very young trees have often given a comparatively poor set of fruit, whereas when older, the same cross-pollinations have resulted in heavy crops of fruit. The comparatively low yields so far obtained from the varieties Baumann's May and Beeve's Heart following compatible pollinations (see Table IX) are probably examples of the above. Both are young trees recently introduced into the experiments. When, however, due allowances have been made for the influences of the above-mentioned and

other environmental factors, it is clear that although they play the larger part, they are not the only cause of the variation. In certain cases it appears that degrees of generational sterility affect the proportion of fruits which set and reach maturity. This naturally will be more evident on the female than on the male side ; for, whereas a moderate or even a fairly high proportion of bad pollen might not detract from the effectiveness of a variety as a pollinator, a similar proportion of sterility among the ovules might result in a lower yield. For example, as shown in Table IX, Black Tartarian " A ", when used as female, has given a low yield in numerous crosses, and it is significant that this variety has a comparatively low proportion of good pollen (see Table VI).

A comparison of Figs. 2 and 3 shows that from a number of compatible pollinations, Black Tartarian " A " (Fig. 2) gave only an average set of 10·3%, whilst Big. Napoleon (Fig. 3) gave 55·9%. Reference to Table IX also shows wide differences in the yield of these varieties. The highest proportion of fruit obtained from Black Tartarian " A " is 21%, whereas from Big. Napoleon over 60%, has frequently been obtained. There seems little doubt that the consistent low yield obtained from Black Tartarian " A " is due to a fairly high proportion of sterility operating on its female side. Although not so wide, similar differences are evident among other varieties.

It is possible in certain cross-pollinations that, in addition to a degree of generational sterility, a proportion of the pollen may also be incompatible, i.e. in such combinations as $S_1S_2 \times S_1S_3$ discussed later in this paper. As a source of differences of yield such a possibility cannot be entirely precluded.

The effect of degrees of generational sterility in the Sweet Cherry and other cultivated fruits is commonly evident in the proportion of good seeds which form. In cherries it is rare for both of the ovules in the ovary to develop and form good seeds ; in certain varieties of plums, however, two seeds commonly form, and as many as 40% have been found to develop two viable seeds (Crane 1920). At the beginning of these experiments the seeds were not always examined, but in recent years they have been recorded in some detail and the results are given in Table III.

The seeds recorded as bad were all very poorly developed, and in most cases little more than an empty testa remained at the time of examination. After the fruits were fully ripe the stones were removed and stratified in pots in sterilized sand, and were kept in a moist condition. Care was taken that they did not become too dry or too wet. The second or third week in the following year the stones were opened and examined ; germination had usually begun by this time. In Table III it will be seen that out of a total of 17,939 stones examined 11,355 seeds were obtained. Only twenty stones contained two seeds each. The seeds recorded as good varied to some extent ; a proportion had a slight depression or sunken area, usually at the apical end. The

TABLE III.

Variety.	Cross-compatible Pollinations.			Seed Content.		
	Flowers.	Fruit.	%	Stones.	Seeds.	%
Baumann's May	69	5	7·2	4	2	50·0
Bedford Prolific	7,969	2,774	34·8	1,782	967	54·3
Belle Agathe	3,240	992	30·6	715	650	90·8
,, de Orleans	3,441	604	17·5	316	67	21·2
Big. Gaucher	125	30	24·0	30	30	100·0
,, Jaboulay	1,331	323	24·2	98	31	31·6
,, Kentish	6,830	1,183	17·3	680	525	77·2
,, Ludwigs	798	150	18·8	108	73	67·6
,, Napoleon	6,172	1,855·	30·0	1,060	745	70·3
,, Schrecken	11,350	2,415	21·3	1,121	707	63·1
Black Circassian	22	8	36·3	8	1	12·5
,, Cluster	307	135	43·9	103	72	69·9
,, Downton	212	55	25·9	33	0	0·0
,, Eagle	2,094	425	20·3	216	47	21·8
,, Elton	233	75	32·2	61	2	3·3
,, Heart	471	107	22·7	9	1	11·1
,, Heart " B "	389	77	19·8	11	3	27·3
,, Oliver	72	46	63·9	26	6	23·1
,, Tartarian " A "	2,452	241	9·8	119	13	10·9
,, Tartarian " B "	802	164	20·4	9	0	0·0
,, Tartarian " D "	658	181	27·5	154	24	15·6
,, Tartarian " E "	331	57	17·2	23	10	43·5
Bohemian Black	5,224	1,231	·23·5	758	601	79·3
Cryall's Seedling	64	33	51·6	31	19	61·3
Early Purple Gean	617	132	21·4	102	24	23·5
Early Rivers	5,373	1,389	25·8	928	607	65·4
Elton Heart	2,508	725	29·0	558	223	39·9
Emperor Francis	5,490	1,193	21·7	738	525	71·1
Florence	1,184	503	42·5	299	231	77·2
Frogmore Early	5,717	1,757	30·5	1,187	608	51·2
Géante de Hedelfingen	3,169	813	25·6	767	688	89·6
Goodneston Black	44	31	70·4	27	28	100·0+
Governor Wood	4,082	1,471	36·0	759	220	28·9
Guigne d'Annonay	1,515	786	51·9	428	153	35·7
Ham Green Black	223	141	63·2	135	110	81·4
Knight's Early Black	838	222	26·4	83	49	59·0
Late Black Big.	3,701	1,121	30·2	691	560	81·0
Monstrueuse de Mezel	1,667	552	33·1	294	229	77·8
Mumford Black	270	48	17·8	10	1	10·0
Noble	3,617	1,042	28·8	855	617	72·1
Noir de Guben	2,008	925	46·2	407	333	81·8
Noir de Schmidt	4,381	780	17·8	449	256	56·9
Ohio Beauty	104	7	6·7	7	7	100·0
Peggy Rivers	950	319	33·5	301	285	94·7
Preserving	114	92	80·7	75	73	97·3
Red Turk	225	84	37·3	12	9	75·0
Roundel Heart	444	150	33·7	107	30	28·0
Smoky Dunn	161	45	27·9	40	8	20·0
Stark's Gold	1,648	377	22·9	350	301	86·0
Turkey Heart	3,387	658	19·4	375	263	70·1
Ursula Rivers	1,344	304	22·5	204	190	93·0
Victoria Black	508	72	14·2	14	11	78·0
Waterloo	1,920	371	19·3	217	127	58·5
Yellow Spanish	31	4	12·9	4	2	50·0

majority were plump and, from external appearances, well developed ; but of the total seeds sown the average germination was only 58·4%.

In a recent paper Tukey (1933) makes the general statement that " Early-ripening varieties of Sweet Cherry should not be used as female parents in the breeding of new varieties. Breeding records involving late-ripening and early-ripening varieties of cherries show no progeny when early-ripening varieties are used as female parents, but do show progeny when used as male parents, indicating that the problem of production of non-viable seed is not genetic." That late-ripening cherries may develop a larger proportion of apparently good seeds may in some cases be true, but even this is not a universal rule, as can be seen from Table IV.

TABLE IV.

Variety	No of Seeds sown	No. germinated	Percentage of germination.
Early Purple Gean	19	18	94·7
Early Rivers	93	62	66·6
Frogmore Early	186	90	48·3
Knight's Early Black	38	16	42·1
Governor Wood	65	25	38·4
Bedford Prolific	258	182	70·5
Big Kentish	113	63	55·7
Big de Schrecken	196	113	57·6
Elton Heart	42	24	57·1
Emperor Francis	82	45	54·8
Géante de Hedelfingen	186	148	74·2
Late Black Big	39	20	51·2
Montrueuse de Mezel	16	9	56·2
Stark's Gold	140	87	62·1
Ursula Rivers	93	89	95·6
Waterloo	81	63	77·7
Big. Gaucher	21	19	93·8
Big. Napoleon	199	157	78·8
Bohemian Black	27	17	62·9
Florence	182	120	65·9
Noble	283	74	26·1
Noir de Schmidt	142	35	24·6
Total	2,483	1,476	59·4

It is also shown in this Table that early-ripening varieties have developed good seeds. Some of these were sown, and the proportion which germinated is shown in Table IV, which includes details of a number of both early and late-ripening varieties. The first five varieties in this Table, i.e. Early Purple Gean to Governor Wood, are early-ripening varieties. At the other extreme, Florence, Big. Gaucher and Noble are late-ripening varieties.

We have previously pointed out that degrees of sterility are more likely to be reflected on the female than on the male side, owing to the fact that there are only two female to almost innumerable male germ-cells in each flower ; and the statement that " The problem of production of non-viable seed is not

genetic " is far too wide an assertion. This statement cannot be accepted as a generalization in the Sweet Cherries any more than it can be accepted and applied to plants in general. On the contrary, our results show that, although sterility is an involved and complex problem, its complexity has a genetic and hereditary basis. The same variety of cherry when used as female and pollinated with different varieties often varies considerably in the proportion of seeds formed. Such differences have varied from 100 to 0% of apparently good seeds ; and since these differences have occurred on trees under identical conditions, and often on the same tree, it is more likely that they are due to genetic causes than directly and entirely to nutritional or other environmental causes.

Among a number of the families raised, albino and albinotic variegated seedlings have occurred. Details are given in Table V (see also Fig. 4). The albinos, and most of the variegated seedlings, die at an early stage, and it is probable, apart from the occurrence of these non-viable seedlings, that genetic factors may in certain cases affect the development of the embryo and give rise to non-viable seeds.

TABLE V.

Parents.		Number of Seeds sown.	Number Germinated.	Percentage Germinated.	Number of Albinos.	Number Variegated.
Big. Gaucher	× Knight's Early Black ..	21	19	90·5	2	- -
,, Napoleon	× Géante de Hedelfingen ..	19	14	73·6	3	---
,, ,,	× Ursula Rivers	20	9	45·0	5	- -
,, ,,	× Ursula Rivers	12	10	83·3	1	–
Florence	× Géante de Hedelfingen ..	53	41	77·3	4	5
,,	× Noir de Schmidt ..	16	9	56·2	2	---
Géante de Hedelfingen	× Big. Jaboulay	26	17	65·4	—	3
,, ,,	× Ursula Rivers	70	40	57·1	13	---
Late Black Big.	× Big.Gaucher	24	7	29·1	2	---
Monstrueuse de Mezel	× Florence	16	9	56·2	---	1
Noble	× Noir de Schmidt	33	11	33·3	2	—
Ursula Rivers	× Géante de Hedelfingen ..	55	35	63·6	9	—
Waterloo	× Florence	35	20	57·1	—	6

As far as the formation and development of fruit is concerned the total results obtained from pollinating Sweet varieties with varieties of the Sour Cherries are not far below those obtained from compatible pollinations of Sweet with Sweet. The results are 20 and 22·5% respectively (see Table II). But where the Duke varieties were used, an appreciably less proportion of fruit developed, i.e. 15·1%. This is undoubtedly attributable to the higher degree of sterility of the Duke Cherries, which results from their hybrid origin and is characteristic of the Duke group. As expressed by aborted pollen grains

and the proportion which germinate, it is shown in Table VI that, although there is a considerable range of variation in the degree of sterility within both the Sweet and Duke Cherries, it is in all cases much higher in the Duke varieties. The pollen germination tests detailed in Table VI were made on agar as a medium, and different concentrations of cane sugar were used, ranging from 5 to 12%. In some cases a slightly higher proportion germinated in the 12% cane sugar, in others the proportion was slightly higher in 5%, but in all cases the differences were very small. In a previous paper (Crane and Lawrence 1929) it was shown that in the reciprocal pollinations, Sour by Sweet and Dukes by Sweet, a much smaller proportion of fruit was obtained, namely 8·1 and 5·3 respectively. The number of flowers used in these pollinations was not very extensive, the actual figures being :—

Sour by Sweet, flowers pollinated 1,144 ; fruits set 93 ;

Dukes by Sweet,   ,,   ,,   2,406 ;   ,,   ,, 129.

Since then, 2,400 additional pollinations have been made, and the total proportion of fruit which set remains approximately the same. Amongst these pollinations, however, considerable variations occurred. A 20% set or over was obtained in a very few cases. The majority gave less than 10%, and a few failed entirely. In the cases of failure the numbers were not high enough to determine whether or not such failure was due to incompatibilty.

We have previously pointed out that the differences obtained from these reciprocal pollinations may arise from difficulties in endosperm formation, as,

TABLE VI.

Varieties.	No of Pollen grains examined.	No. of Pollen grains germinated.	No. of aborted Pollen grains.	Percentage of germination
Bedford Prolific	1,898	1,845	53	97·2
Belle Agathe	1,112	959	153	86·2
Big. Napoleon	1,214	905	309	74·6
Big. Schrecken	921	623	298	67·6
Black Eagle	1,908	1,473	435	77·2
Black Tartarian " A "	1,058	583	475	55·1
Early Rivers	1,639	1,345	304	82·6
Emperor Francis	1,481	1,273	208	85·9
Governor Wood	1,084	980	104	90·4
Guigne d'Annonay	590	563	27	95·4
Knight's Early Black	618	531	87	85·9
Noir de Schmidt	1,507	746	761	49·2
Waterloo	1,098	936	162	85·2
Duke Cherries :				
Archduke	972	78	714	9·8
Empress Eugenie	1,213	331	882	27·3
Late Duke	894	132	762	14·8
May Duke	1,595	223	1,372	13·8
Reine Hortense	1,359	451	908	33·2
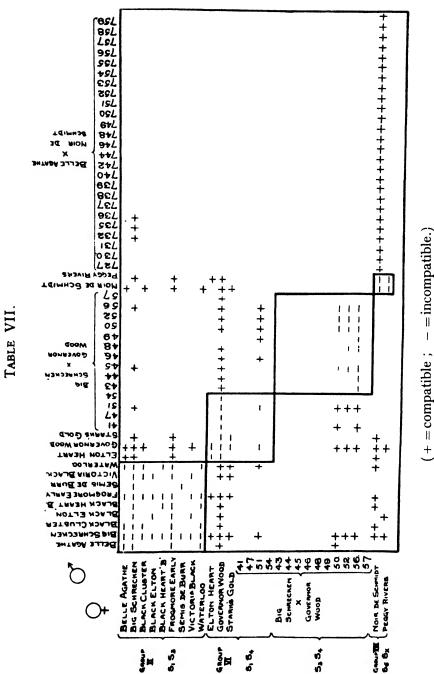
following triple fusion, the endosperm of the tetraploid Duke and Sour Cherries when pollinated by the diploid Sweet Cherries will be pentaploid, or approximately so. But in the reciprocal pollinations, where the Sweet Cherries are used as females, the endosperm will be tetraploid. Possibly the endosperm of pentaploid constitution may have greater developmental difficulties than the tetraploid endosperm, and this may be the cause of the lower yield where the tetraploid Sour Cherries are used as females, and the differences in the proportion of fruit set in the reciprocal pollinations between these groups.

THE VARIETAL BEHAVIOUR AND HEREDITARY BASIS OF INCOMPATIBILITY.

In a previous paper (Crane and Lawrence 1930) we published some preliminary results and concluded that the hereditary basis of incompatibility in the Sweet Cherry was amenable to the oppositional factor hypothesis advanced by East and Mangelsdorf (1925) from their extensive work on Nicotiana, and subsequently found to apply to many other plants. We have continued to make a factorial analysis of the hereditary and varietal behaviour of incompatibility by determining the behaviour of pollinations among the progeny of inter-group crosses, and from pollinations between them and their parents. Some of the results obtained are given in Table VII. They show that the progeny raised from Belle Agathe (Group II) crossed Noir de Schmidt (Group VIII) are all effective on their male parent, whilst the progeny from Big. de Schrecken (Group II) crossed Governor Wood (Group V) form two intra-sterile, inter-fertile groups, one of which fails with its male parent. Details of the pollinations recorded in Table VII are: compatible pollinations, flowers 5,134, fruits set 1,153, $= 22 \cdot 4\%$; incompatible pollinations, flowers 5,930, fruits set 5, $= 0 \cdot 08\%$.

Incompatibility is determined by genetic factors which control pollen-tube growth, and the essential feature of the genetic behaviour of incompatibility is that under normal conditions pollen cannot function in the style of a plant carrying the same factors as the pollen. These factors form a multiple series of allelomorphs, and any two of them may be carried by a given plant. Thus, following East's and Mangelsdorf's terminology, effective and non-effective pollinations arise as follows :—

			$S_1S_2 \times S_1S_2$		$S_1S_2 \times S_3S_4$		$S_1S_2 \times S_1S_3$	
Male germ cells	$=$		S_1	S_2	S_3	S_4	S_1	S_3
Female tissue { Female germ-cells.		S_1	fail	fail	S_1S_3	S_1S_4	fail	S_1S_3
		S_2	fail	fail	S_2S_3	S_2S_4	fail	S_2S_3

TABLE VII.

(+ = compatible ; − = incompatible.)

The offspring from $S_1S_2 \times S_3S_4$ will fall into four incompatible groups and those from $S_1S_2 \times S_1S_3$ into two. All the seedlings in the former cross will be reciprocally compatible with their parents, as in the family raised from Belle Agathe × Noir de Schmidt. In the latter cross, however, the S_1S_3 group will be reciprocally incompatible with its male parent, as occurred in the family from Big. de Schrecken × Governor Wood, for they carry the same factors. All other combinations between parents and the F_1 generation will be effective, as they differ in at least one respect. A number of other seedlings, raised from different inter-group crosses, have been used in similar tests. They have usually comprised small units of about six sister-seedlings, and consequently the details could be given only at considerable length ; but in all cases the results obtained have been in agreement with the above factorial scheme. These recent results, therefore, substantiate our earlier conclusions, and they are also beginning to show the genetic constitution and relationship of the established varieties investigated and the groups to which they belong. Amongst the Sweet Cherries in .cultivation we have tested 66 varieties. They fall into 11 groups, and in addition 21 varieties remain independent (see Table I). Therefore, at least 32 different combinations and a minimum of 9 incompatibility factors are necessary to interpret the results so far obtained.

As shown in Table IX. a few fruits have been obtained both from selfing and from cross-incompatible pollinations, but very few of these fruits have developed perfect seeds, and the majority have been non-viable. Possibly some of these fruits are the results of accident, but some have set under very stringent conditions, which suggests that, as a rarity, a pollen-tube travels the full length of the style and effects fertilization. One of the seedlings which arose in these experiments in this way, No. 500, came from Big. Frogmore × Big. de Schrecken, co-incompatibles in Group II. The results of pollinations made between this seedling and its parents are as follows :—

Big. Frogmore × No. 500 (1932)	..	Flowers 66	Fruits 0
,, ,, × ,, ,, (1933)	..	,, 16	,, 0
		Total	,, 82	,, 0
Big. de Schrecken × No. 500 (1932)	..	,, 49	,, 0
. ,, ,, ,, × ,, ,, (1933)	..	,, 30	,, 0
		Total .	,, 79	,, 0

We have provisionally designated the genetic constitution of Group II as S_1S_2. Therefore, if fertilization occurred within this group it would give rise either to plants of the same constitution as the parents, e.g. S_1S_2, or to homozygous

plants, S_1S_1 or S_3S_3. Those of the same constitution would reciprocally fail with their parents. The homozygous derivatives would be effective when used as females, but would also fail when used as males in pollinations with their parents. The results of the pollinations made between Big. Frogmore, Big. de Schrecken and seedling No. 500 are therefore in agreement with the view that the seedling arose from fertilization between co-incompatible varieties. Seedling No. 500, although of healthy growth, lacks vigour and is only about half the size of trees of the same age raised from parents which are normally compatible.

Among the established varieties of the Sweet Cherry investigated, cross-incompatibility has always been reciprocally expressed ; that is to say, if A fails with B, it has always followed that B failed with A. It is theoretically possible for varieties to originate which would be compatible in one direction of a cross and fail in the other, e.g. homozygous varieties such as S_1S_1, etc. ; but if the lack of vigour of seedling No. 500 is also characteristic of such individuals, they are unlikely to be grown commercially or to receive varietal recognition.

VARIETAL CONFUSION.

Considerable confusion prevails in the varietal nomenclature of cherries ; different individuals are frequently distributed under one varietal name, and to find an individual under two or more names is also common. Many of the varieties we have investigated are widely cultivated and are to be found in almost any district where cherries are grown ; but the cultivation of others is more restricted and some appear to be confined to local areas.

As far as we have been able to determine, all the varieties we have investigated are distinct individuals, but the identity of some is obscure and still under investigation. It will be noted in Tables I and IX that four individuals, designated " A ", " B ", " D " and " E ", bear the name of Black Tartarian. All these individuals have black-coloured fruits, but they differ in such characters as the size, shape and pigmentation of leaves and flowers, and are quite distinct. Black Heart is also different from Black Heart " A ". Trees referred to in a previous paper as Windsor " A " and Mezel " A " were purchased as Windsor and Mezel respectively, but both were eventually found to be Big. Napoleon. Of other trees obtained under the name of Monstrueuse de Mezel, some have completely failed on Géante de Hedelfingen, while others have effectively pollinated this variety. They therefore appear to be different individuals. We have used comparatively few varieties of the Sour Cherries in pollinations with Sweet Cherries, but even in the few used, similar difficulties have occurred. Trees obtained under the name of Morello have differed in minor respects, and,

as detailed in an earlier report, Kentish Red and Kentish Red " A " are distinct ; the former is self-incompatible and has comparatively long pedicels, whilst Kentish Red " A " is self-compatible and has short pedicels.

We are indebted to Mr. N. H. Grubb for a number of the varieties we have used, particularly the local varieties, and also for much valuable information concerning the identity of varieties, obtained during visits to the East Malling Research Station over a period of years.

THE INTERPLANTING OF VARIETIES.

Although many factors such as disease, nutrition, climatic conditions, etc., affect the initial setting of the fruit and the ultimate yield, the results of the present experiments show that effective pollination and fertilization is a first essential for the formation and development of the fruits of cherries.   Therefore, since self-incompatibility appears to be general throughout the Sweet Cherries and cross-incompatibility common, it is important to ensure that adequate provision is made for effective cross-pollination.

No variety of the Sweet Cherry should be planted in complete isolation either as single trees in private gardens or as large blocks in commercial plantations ; and care should be taken to interplant varieties which are known to be compatible and which flower at the same time.

Knowledge of the relative flowering times of cherries in cultivation is far from complete, and nothing appears to be known precisely about alleged fluctuations of this character ; but two recent publications, Hooper (1932) and Beakbane, Chapelow and Grubb (1935), deal specially with this subject and provide much valuable information.   The latter investigators state that early and late blossoming varieties do not overlap enough in season for effective cross-pollination, and in planning a cherry orchard, this fact, as well as that of cross-incompatibility, must be allowed for.   They also show that varieties differ in the length of their blossoming period, and also in the relative position of their times of full bloom.

The relative flowering times as given by Hooper do not in all cases agree with those given by Beakbane and her collaborators, indeed in some cases they differ considerably.   The records of the blossoming period by Beakbane *et al.* were made at the East Malling Research Station from two sets of trees, one planted in 1921 and the other in 1926, and their average period of blossoming and time of full bloom is given for six and four years respectively.   In Table VIII we have attempted, as far as the details will allow, to co-ordinate their results with our work on incompatibility.   In this Table the relative time of full bloom is given, and co-incompatible varieties and the group to which they belong are indicated by the Roman numeral.

TABLE VIII.

Varieties of the Sweet Cherry, in order of time of full bloom.

Ham Green Black
Early Cluster
Guigne d'Annonay (XI)
Noir de Guben
Cryall's Seedling (XI)
Red Turk (IX)
Baumann's May (I)   } April 21st to 27th.
Black Tartarian " E "
Turkey Heart (V)
Bedford Prolific (I)
Black Heart " B " (II)

Black Oliver
Early Rivers (I)
Belle Agathe (II)
Big. Ludwigs (IV)
Black Eagle (I)   } April 27th to May 1st.
Waterloo (II)
Preserving
Ohio Beauty (III)
Knight's Early Black (I)
Black Circassian (I)
Roundel Heart (I)
Elton Heart (VI)

Black Cluster (II)   May 1st to 8th.
Victoria Black (II)
Governor Wood (VI)
Big. Kentish (IV)
Frogmore Early (II)
Large Black

In addition to the varieties of Sweet Cherry given in this Table, Hooper gives the following : —
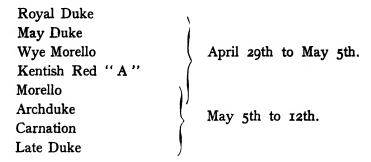
Early flowering : Early Purple Gean, Goodneston Black.

Mid-season flowering : Monstrueuse de Mezel (VIII), Big. Jaboulay (X), Noir de Schmidt (VIII), Big. de Schrecken (II).

Late flowering : Big. Napoleon (III), Florence. Noble, Géante de Hedel-fingen (VII).

(The Roman numerals show co-incompatibles, as in the above Table.)

In their report, Beakbane *et al.* also give the time of full bloom of a number of varieties of the Sour and Duke Cherries as follows:—

Royal Duke   ⎫

May Duke

Wye Morello   ⎬   April 29th to May 5th.

Kentish Red " A "

Morello   ⎫

Archduke

Carnation   ⎬   May 5th to 12th.

Late Duke   ⎭

Hooper gives Empress Eugenie and Flemish Red as late flowering varieties.

ACKNOWLEDGMENTS.

SUMMARY.

In this paper the results obtained from over 236,000 pollinations of cherries are described. In the Sweet Cherry self-incompatibility was found to be the rule, cross-incompatibility common and always reciprocally expressed. Out of a total of 66 varieties investigated, 45 were found to belong to 11 incompatible groups, within which all self- and cross-pollinations fail.

An analysis of investigations with seedlings shows that incompatibility in the Sweet Cherry is an orderly phenomenon, and the results, as far as they go, are in agreement with the oppositional factor hypothesis advanced by East and Mangelsdorf for Nicotiana.

Incompatibility in the Sweet Cherry is expressed by the young fruits ceasing to grow and falling from the tree at an early stage. It is due to the pollen-tubes being arrested in their growth down the stylar tissue, hence fertilization cannot take place and the fruit fails to develop.

Degrees of generational sterility occur and are expressed by aborted pollen, imperfectly developed or non-viable seeds and occasionally by differences in the proportion of fruits which set and reach maturity.

Since cross-incompatibility is of common occurrence in the Sweet Cherry, the interplanting of suitable varieties, so that provision is made for effective cross-pollination, is of the first importance. When making the provision both compatibility and the time of flowering of varieties must be considered.

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TABLE IX.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.	Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
BAUMANN'S MAY.				White Big. ..	125	41	33
Selfed	89	0	0	Yellow Spanish	86	24	28
Bedford Prolific	13	0	0	Carnation*	32	2	6
Early Rivers	78	1	1	May Duke*	27	13	48
Ronald's Heart	45	0	0	Prunus decumana	12	1	8
Big. Schrecken	44	2	4				
Black Elton	6	1	17	**BEEVE'S HEART.**			
West Midlands Big.	19	2	10	Selfed	22	0	0
				Big. Napoleon	30	1	3
BEDFORD PROLIFIC.							
				BELLE AGATHE.			
Selfed	2,020	0	0	Selfed	1,281	0	0
Baumann's May	161	1	1	Big Schrecken	923	0	0
Black Downton	361	0	0	Black Cluster	35	1	2
Black Eagle	313	0	0	Frogmore Early	897	0	0
Black Tartarian A	207	0	0	Waterloo	550	1	0
Black Tartarian B	158	0	0	Bedford Prolific	248	124	50
Early Rivers	1072	0	0	Belle d'Orléans	158	24	15
Knight's Early Black	354	0	0	Big Jaboulay	47	31	66
Leicester Black	44	1	2	Big. Kentish	152	20	13
Roundel Heart	428	0	0	Big. Ludwig's	138	14	10
Belle Agathe	424	102	24	Big. Napoleon	131	64	49
Belle d'Orléans	163	65	40	Black Eagle	226	17	7
Big Jaboulay	40	4	10	Black Tartarian A	32	17	53
Big. Ludwigs	45	5	11	Black Tartarian B	70	12	17
Big. Napoleon	1,077	374	35	Black Tartarian E	52	2	4
Big. Schrecken	414	134	32	Bohemian Black	316	59	19
Black Cluster	273	72	26	Early Purple Gean	24	7	29
Black Elton	428	61	14	Early Rivers	51	9	18
Black Heart B	168	61	36	Elton Heart	224	33	15
Black Oliver	229	66	29	Emperor Francis	221	61	28
Black Tartarian E	98	53	54	Géante de Hedelfingen	57	23	40
Bohemian Black	95	66	69	Governor Wood	49	23	47
Early Purple Gean	41	17	41	Knight's Early Black	90	16	18
Elton Heart	26	21	81	Late Black Big.	119	16	13
Emperor Francis	671	231	34	Monstrueuse de Mezel B	132	29	22
Frogmore Early	501	192	38	Noble	196	22	11
Géante de Hedelfingen	39	6	15	Noir de Guben	166	59	35
Goodneston Black	154	115	75	Noir de Schmidt	146	74	51
Governor Wood	249	162	65	Roundel Heart	18	8	44
Ham Green Black	34	18	53	Turkey Heart	177	12	7
Late Black Big.	118	58	49	White Big. ..	177	62	35
Monstrueuse de Mezel B	184	85	46	May Duke*	197	28	14
Noble	36	23	64	Prunus decumana	18	7	39
Noir de Guben	40	4	10				
Noir de Schmidt	128	25	19	**BELLE D'ORLÉANS.**			
Ohio Beauty	57	24	42	Selfed	3081	1	0
Peggy Rivers	71	29	41	Bedford Prolific	28	8	29
Red Turk	139	67	48	Belle Agathe	59	9	15
Semis de Burr	33	9	27	Big. Gaucher	73	12	16
Smoky Dunn	46	20	43	Big. Jaboulay	89	29	33
Stark's Gold	537	169	31	Big. Kentish	138	12	9
Turkey Heart	131	93	71	Big. Napoleon	97	18	18
Ursula Rivers	304	169	56	Big. Schrecken	775	45	5
Victoria Black	64	7	11	Black Cluster	83	18	22
Waterloo	273	96	35				

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Black Tartarian A ..	46	18	39
Bohemian Black	103	8	8
Cryall's Seedling	198	43	22
Early Rivers	67	13	19
Elton Heart	221	54	24
Emperor Francis	239	4	17
Florence	79	15	19
Frogmore Early	12	2	17
Governor Wood	98	27	27
Guigne d'Annonay	125	35	28
Knight's Early Black ..	28	13	46
Large Black	159	56	35
Late Black Big.	198	23	12
Monstrueuse de Mezel B ..	59	17	29
Mumford Black	196	53	27
Noble	44	11	25
Noir de Guben	108	32	30
Noir de Schmidt	15	6	40
Preserving	122	23	19

BIGARREAU GAUCHER.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*103*	*0*	*0*
Big. Napolean	7	3	42
Big. Schrecken	70	6	9
Knight's Early Black ..	48	21	44

BIGARREAU JABOULAY.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*709*	*1*	*0*
Black Tartarian D ..	*24*	*0*	*0*
Bedford Prolific	52	32	61
Belle Agathe	87	39	45
Belle d'Orléans	57	6	10
Big. Napoleon	37	8	22
Black Heart B	37	9	24
Black Tartarian B	18	13	72
Bohemian Black	76	10	13
Early Purple Gean	48	16	33
Early Rivers	105	14	13
Emperor Francis	30	4	13
Florence	126	9	7
Frogmore Early	24	18	75
Géante de Hedelfingen ..	37	8	22
Knight's Early Black ..	39	9	23
Late Black Big.	93	21	22
Leicester Black	14	2	14
Noble	.49	15	31
Noir de Guben	138	34	25
Noir de Schmidt	82	24	29
Peggy Rivers .. .: ..	74	7	9
Roundel Heart	34	13	38
Waterloo	21	6	28
White Big.	29	3	10
Archduke*	24	0	0
May Duke*	202	3	1
Royal Duke*	134	12	9
Kentish Red*	16	8	50
Reine Hortense*	26	2	8

BIGARREAU KENTISH.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*1,152*	*0*	*0*
Big. Ludwig's	*145*	*0*	*0*
White Big.	*608*	*0*	*0*
Yellow Spanish	*49*	*0*	*0*
Baumann's May	119	14	12
Bedford Prolific	275	23	8
Belle Agathe	89	11	12
Big. Gaucher	37	6	16
Big. Jaboulay	30	9	30
Big. Napoleon	1,189	215	18
Big. Schrecken	214	31	14
Black Downton	109	6	5
Black Eagle	72	16	22
Black Elton	123	12	10
Black Heart B	71	15	21
Black Oliver	98	27	31
Black Tartarian D ..	236	46	19
Bohemian Black	943	93	10
Early Rivers	228	37	16
Florence	140	21	15
Géante de Hedelfingen ..	228	28	12
Goodneston Black	241	60	24
Guigne d'Annonay	930	184	20
Ham Green Black	100	18	18
Mumford Black	127	38	30
Noir de Guben	25	8	32
Noir de Schmidt	287	60	21
Peggy Rivers	42	9	21
Red Turk	102	15	15
Ronald's Heart	67	13	19
Smoky Dunn	142	5	3
Turkey Heart	86	27	31
Ursula Rivers	385	106	27
Victoria Black	95	4	4
Archduke*	156	26	17
Late Duke*	91	21	23
May Duke*	235	3	1
Royal Duke*	37	5	13
Carnation*	204	3	1
Kentish Red*	68	6	9
Morello*	491	26	5
Wye Morello*	61	9	15
Prunus decumana	154	42	27

BIGARREAU LUDWIG'S.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*209*	*0*	*0*
Big. Kentish	*66*	*0*	*0*
White Big.	*54*	*0*	*0*
Bedford Prolific	79	15	19
Belle d'Orléans	23	6	26
Big. Jaboulay	44	15	34
Big. Napoleon	61	18	29
Black Cluster	97	7	7
Elton Heart	51	11	21
Emperor Francis	114	17	15
Knight's Early Black ..	128	23	18

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Monstrueuse de Mezel B ..	27	6	22
Noble	7	1	14
Noir de Schmidt	13	4	31
Waterloo	154	27	18
BIGARREAU NAPOLEON.			
Selfed ..	1,429	2	0
Emperor Francis ..	1,350	1	0
Ohio Beauty ..	27	0	0
Baumann's May ..	169	30	18
Bedford Prolific ..	114	56	49
Belle Agathe ..	186	77	41
Belle d'Orléans ..	186	63	34
Big. Gaucher ..	192	15	8
Big. Jaboulay ..	41	13	32
Big. Kentish ..	98	38	39
Big. Ludwig's ..	222	19	8
Big. Schrecken ..	143	76	53
Black Cluster ..	33	20	61
Black Downton ..	65	17	26
Black Eagle ..	170	44	26
Black Elton ..	98	25	25
Black Heart B ..	119	50	42
Black Oliver ..	90	12	13
Black Tartarian A ..	50	32	64
Black Tartarian B ..	71	47	66
Black Tartarian D ..	174	44	25
Bohemian Black ..	50	33	66
Cryall's Seedling ..	61	21	34
Early Purple Gean ..	54	33	61
Early Rivers ..	77	44	57
Elton Heart ..	20	4	20
Florence	104	28	27
Frogmore Early ..	82	21	26
Géante de Hedelfingen ..	202	74	37
Goodneston Black ..	146	31	21
Governor Wood ..	65	35	54
Guigne d'Annonay ..	422	137	31
Ham Green Black ..	154	26	17
Knight's Early Black ..	92	41	44
Large Black ..	50	5	10
Late Amber ..	42	6	14
Late Black Big. ..	203	73	36
Monstrueuse de Mezel B ..	153	43	28
Mumford Black ..	44	6	14
Noble	301	48	16
Noir de Guben ..	44	17	39
Noir de Schmidt ..	483	151	31
Peggy Rivers ..	51	10	20
Preserving ..	80	4	5
Red Turk ..	143	47	33
Ronald's Heart ..	30	2	7
Roundel Heart ..	36	19	53
Semis de Burr ..	31	3	10
Smoky Dunn ..	43	4	9
Stark's Gold ..	295	51	18
Turkey Heart ..	88	9	10

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Ursula Rivers	109	40	37
Victoria Black	36	4	11
Waterloo	56	36	65
White Big. ..	75	32	42
Yellow Spanish ..	29	2	7
Archduke* ..	13	2	15
Empress Eugenie* ..	31	24	77
Late Duke* ..	22	4	18
May Duke* ..	16	11	69
Royal Duke* ..	26	10	38
Carnation* ..	75	4	5
Kentish Red* ..	20	11	55
Wye Morello* ..	28	6	21
BIGARREAU SCHRECKEN.			
Selfed ..	3,286	0	0
Belle Agathe ..	961	3	0
Black Cluster ..	161	0	0
Black Elton ..	171	1	0
Black Heart B ..	103	1	1
Frogmore Early ..	2,884	1	0
Semis de Burr ..	32	0	0
Victoria Black ..	162	0	0
Waterloo ..	207	1	0
Baumann's May ..	58	6	10
Bedford Prolific ..	1,073	203	20
Beeve's Heart ..	49	3	6
Belle d'Orléans ..	263	71	27
Big. Jaboulay ..	246	71	28
Big. Kentish ..	644	94	15
Big. Ludwig's ..	419	50	12
Big. Napoleon ..	596	160	27
Black Downton ..	50	2	4
Black Eagle ..	117	42	36
Black Heart ..	109	46	42
Black Oliver ..	122	9	7
Black Tartarian B ..	264	57	22
Black Tartarian E ..	60	28	47
Bohemian Black ..	173	29	17
Cryall's Seedling ..	327	45	14
Early Purple Gean ..	429	114	26
Early Rivers ..	1,069	283	26
Elton Heart ..	100	26	26
Emperor Francis ..	552	103	19
Florence	106	32	30
Géante de Hedelfingen ..	146	31	22
Goodneston Black ..	175	20	11
Governor Wood ..	245	53	21
Guigne d'Annonay ..	71	29	41
Ham Green Black ..	29	10	35
Knight's Early Black ..	195	59	30
Late Amber ..	56	11	20
Late Black Big. ..	286	55	19
Leicester Black ..	65	8	12
Monstrueuse de Mezel B ..	473	98	21
Mumford Black ..	122	4	3
Noble	43	15	35

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Noir de Guben	501	162	32
Peggy Rivers	96	7	7
Preserving	164	8	5
Red Turk	54	4	7
Roundel Heart	151	66	44
Smoky Dunn	162	39	24
Stark's Gold	280	50	18
Turkey Heart	220	44	20
Ursula Rivers	46	8	17
White Big.	694	35	5
Yellow Spanish	79	10	13
Late Duke*	111	22	20
Royal Duke*	671	44	6
Kentish Red*	95	24	25
Morello*	219	57	26
Chinese Early*	101	3	3
Prunus decumana	39	17	43

BLACK CIRCASSIAN.

Selfed	*122*	*0*	*0*
Big. Napoleon	22	8	36

BLACK CLUSTER.

Selfed	*138*	*0*	*0*
Big. Schrecken	*36*	*0*	*0*
Frogmore Early	*14*	*0*	*0*
Victoria Black	*82*	*0*	*0*
Waterloo	*52*	*0*	*0*
Bedford Prolific	31	13	42
Big. Kentish	28	18	64
Big. Napoleon	41	6	15
Early Purple Gean	11	9	82
Early Rivers	44	43	98
Governor Wood	76	10	13
Noir de Schmidt	36	11	30
Turkey Heart	40	25	62

BLACK DOWNTON.

Selfed	*34*	*0*	*0*
Bedford Prolific	*65*	*0*	*0*
Big. Kentish	56	10	18
Big. Napoleon	156	45	29

BLACK EAGLE.

Selfed	*942*	*0*	*0*
Bedford Prolific	*228*	*0*	*0*
Black Tartarian A	*196*	*0*	*0*
Black Tartarian B	*46*	*0*	*0*
Early Rivers	*47*	*0*	*0*
Knight's Early Black	*111*	*0*	*0*
Roundel Heart	*146*	*0*	*0*
Belle Agathe	290	34	12
Belle d'Orléans	39	6	15
Big. Jaboulay	47	21	45
Big. Kentish	23	20	87
Big. Ludwig's	122	16	13

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Big. Napoleon	41	10	24
Big. Schrecken	183	21	11
Black Cluster	75	7	9
Black Heart B	68	18	26
Black Tartarian D	19	6	31
Bohemian Black	80	12	15
Early Purple Gean	185	67	36
Elton Heart	158	50	32
Emperor Francis	129	9	7
Florence	34	10	29
Frogmore Early	45	9	20
Géante de Hedelfingen ..	20	6	30
Governor Wood	84	28	33
Late Black Big.	97	17	17
Monstrueuse de Mezel B ..	35	12	34
Noble	43	8	19
Noir de Schmidt	29	9	31
Ursula Rivers	52	12	23
Waterloo	196	17	9
Archduke*	49	4	8
May Duke*	69	8	12
Royal Duke*	51	11	21
Morello*	50	13	26

BLACK ELTON.

Selfed	*165*	*0*	*0*
Big. Schrecken	*45*	*1*	*2*
Frogmore Early	*107*	*0*	*0*
Victoria Black	*30*	*0*	*0*
Big. Napoleon	79	39	48
Black Downton	130	25	19
Red Turk	24	11	46

BLACK HEART.

Selfed	*82*	*0*	*0*
Big. Schrecken	69	17	25
Early Rivers	131	18	14
Elton Heart	32	7	22
Frogmore Early	177	58	33
Morello*	62	7	11

BLACK HEART B.

Selfed	*93*	*0*	*0*
Big. Schrecken	*37*	*0*	*0*
Frogmore Early	*58*	*0*	*0*
Waterloo	*18*	*0*	*0*
Big. Kentish	101	35	35
Big. Napoleon	80	10	12
Black Eagle	24	3	12
Guigne d'Annonay	87	20	23
Noble	97	9	9

BLACK OLIVER.

Selfed	*77*	*0*	*0*
Bedford Prolific	34	15	44
Big. Schrecken	24	13	54
Black Elton	14	8	30

* Indicates tetraploid varieties.

BLACK TARTARIAN A.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*1,776*	0	0
Bedford Prolific	65	0	0
Black Eagle	471	0	0
Black Tartarian B	138	0	0
Early Rivers	797	0	0
Knight's Early Black	31	0	0
Roundel Heart	71	0	0
Belle Agathe	509	27	5
Belle d'Orléans	76	7	9
Big. Napoleon	332	26	8
Big. Schrecken	88	13	15
Black Cluster	58	4	7
Black Tartarian D	37	8	22
Black Tartarian E	170	30	18
Emperor Francis	206	17	8
Frogmore Early	277	21	7
Governor Wood	212	29	14
Late Black Big.	182	27	15
Noble	66	12	18
Turkey Heart	150	13	9
Waterloo	89	7	8

BLACK TARTARIAN B.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*458*	0	0
Bedford Prolific	*119*	0	0
Black Eagle	*24*	0	0
Black Tartarian A	*40*	0	0
Early Rivers	*656*	0	0
Knight's Early Black	*57*	0	0
Roundel Heart	*63*	0	0
Belle Agathe	24	6	25
Big. Jaboulay	13	8	61
Big. Napoleon	145	17	12
Big. Schrecken	208	31	15
Black Tartarian E	45	5	11
Elton Heart	102	31	30
Emperor Francis	20	4	20
Frogmore Early	118	23	19
Noir de Guben	58	20	34
Turkey Heart	20	7	35
Waterloo	49	12	24

BLACK TARTARIAN D.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*115*	0	0
Big. Jaboulay	*106*	0	0
Baumann's May	40	5	12
Big. Kentish	41	20	49
Big. Napoleon	26	8	31
Black Heart B	44	17	39
Black Oliver	137	18	13
Florence	23	2	9
Frogmore Early	27	15	56
Goodneston Black	139	32	24
Monstrueuse de Mezel	42	23	55
Noir de Schmidt	32	12	37

* Indicates tetraploid varieties,

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Red Turk	51	22	46
Smoky Dunn	56	7	12

BLACK TARTARIAN E.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*303*	0	0
Belle Agathe	41	16	39
Big. Napoleon	38	6	16
Black Tartarian A	86	8	9
Black Tartarian B	15	5	33
Early Rivers	64	10	16
Frogmore Early	17	1	6
Governor Wood	43	5	12
Noir de Schmidt	27	2	7

BOHEMIAN BLACK.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	*1,013*	3	0
Late Black Big.	*606*	2	0
Turkey Heart	*586*	2	0
Bedford Prolific	202	120	56
Belle Agathe	132	23	17
Belle d'Orléans	165	29	18
Big. Jaboulay	325	61	19
Big. Kentish	63	14	22
Big. Ludwig's	198	41	21
Big. Napoleon	355	66	19
Big. Schrecken	261	101	39
Black Eagle	109	21	19
Black Heart B	441	112	25
Black Tartarian A	43	21	49
Black Tartarian D	67	13	19
Cryall's Seedling	118	4	3
Early Purple Gean	169	22	13
Early Rivers	201	90	45
Elton Heart	36	7	19
Emperor Francis	388	66	17
Florence	174	21	12
Frogmore Early	388	80	21
Géante de Hedelfingen	37	29	78
Governor Wood	227	27	12
Guigne d'Annonay	45	7	16
Knight's Early Black	166	48	29
Large Black	34	9	26
Mumford Black	81	8	10
Noble	34	6	17
Noir de Guben	64	20	31
Noir de Schmidt	530	95	18
Peggy Rivers	84	19	23
Preserving	40	5	12
Roundel Heart	57	12	21
Stark's Gold	20	5	25
Ursula Rivers	35	11	31
Waterloo	35	19	54
Archduke*	34	8	23
May Duke*	108	12	11
Royal Duke*	119	24	20
Carnation*	326	20	6
Kentish Red*	461	103	22

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Morello*	52	14	27
Reine Hortense*	35	7	20
Prunus decumana	80	14	17

CRYALL'S SEEDLING.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	124	0	0
Guigne d'Annonay	14	0	0
Big. Napoleon	15	10	67
Big. Schrecken	26	6	23
Peggy Rivers	11	7	64
Turkey Heart	12	10	83

EARLY CLUSTER.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	41	0	0

EARLY PURPLE GEAN.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	249	0	0
Big. Kentish	87	21	24
Big. Napoleon	119	18	15
Big. Schrecken	131	27	21
Early Rivers	161	35	22
Frogmore Early	4	1	25
Governor Wood	61	10	16
Late Black Big.	14	9	74
Noble	40	11	27

EARLY RIVERS.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	1,340	0	0
Baumann's May	68	1	3
Bedford Prolific	960	1	0
Black Circassian	43	0	0
Black Downton	97	0	0
Black Eagle	590	2	0
Black Tartarian A	235	0	0
Black Tartarian B	639	0	0
Knight's Early Black	339	0	0
Roundel Heart	204	0	0
Belle Agathe	122	21	17
Belle d'Orléans	36	4	11
Big. Jaboulay	56	19	34
Big. Kentish	120	17	14
Big. Napoleon	392	87	22
Big. Schrecken	280	40	14
Black Cluster	494	59	12
Black Elton	258	103	40
Black Heart B	391	124	32
Black Oliver	163	58	56
Black Tartarian D	56	17	30
Bohemian Black	118	24	20
Early Cluster	29	3	10
Early Purple Gean	43	20	46
Emperor Francis	62	12	19
Florence	158	13	8
Frogmore Early	214	34	16
Géante de Hedelfingen	26	16	61

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Goodneston Black	84	31	37
Governor Wood	316	80	25
Guigne d'Annonay	57	25	44
Ham Green Black	44	25	57
Late Black Big.	82	28	35
Mumford Black	55	8	14
Noble	141	31	22
Noir de Schmidt	97	43	44
Ohio Beauty	44	6	14
Peggy Rivers	133	58	44
Red Turk	127	92	88
Smoky Dunn	190	61	32
Stark's Gold	144	33	23
Turkey Heart	328	45	14
Ursula Rivers	224	56	25
Victoria Black	130	65	50
Waterloo	42	22	52
White Big.	127	18	14
May Duke*	170	16	9
Late Duke*	162	31	17
Morello*	75ª	2	3

ELTON HEART.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	429	0	0
Governor Wood	113	0	0
Bedford Prolific	475	68	14
Belle d'Orléans	58	25	43
Big. Jaboulay	71	21	29
Big. Napoleon	178	87	49
Big. Schrecken	299	107	36
Black Heart	264	69	26
Black Tartarian A	35	16	46
Early Purple Gean	49	36	75
Early Rivers	106	9	8
Emperor Francis	68	28	41
Florence	269	60	22
Frogmore Early	143	46	32
Géante de Hedelfingen	199	41	21
Guigne d'Annonay	87	29	33
Knight's Early Black	46	13	28
Noble	51	23	45
Peggy Rivers	23	15	65
Turkey Heart	87	32	37
Late Duke*	80	4	5
May Duke*	150	10	7
Royal Duke*	44	15	34
Wye Morello*	115	35	30

EMPEROR FRANCIS.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	2,109	5	0
Big. Napoleon	1,516	0	0
Bedford Prolific	304	100	33
Belle Agathe	189	73	39
Belle d'Orléans	62	14	23
Big. Jaboulay	50	16	32
Big. Kentish	77	9	12

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.	Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Big. Ludwig's	119	13	11				
Big. Schrecken	392	115	29	**FROGMORE EARLY.**			
Black Cluster	55	19	34	*Selfed*	*1,201*	*0*	*0*
Black Eagle	369	76	21	*Belle Agathe*	*281*	*0*	*0*
Black Heart B	70	7	10	*Big. Schrecken*	*1,205*	*1*	*0*
Black Tartarian A	121	29	24	*Black Cluster*	*92*	*1*	*1*
Black Tartarian B	75	27	36	*Black Elton*	*256*	*1*	*0*
Black Tartarian E	31	16	52	*Black Heart B*	*108*	*0*	*0*
Bohemian Black	184	52	28	*Victoria Black*	*122*	*0*	*0*
Cryall's Seedling	65	2	3	*Waterloo*	*406*	*1*	*0*
Early Purple Gean	233	60	26	Baumann's May	80	36	45
Early Rivers	205	72	35	Bedford Prolific	129	59	46
Elton Heart	290	56	19	Beeve's Heart	28	2	7
Florence	301	28	9	Belle d'Orléans	78	21	27
Géante de Hedelfingen	27	2	7	Big Gaucher	37	7	19
Governor Wood	173	43	25	Big Jaboulay	56	7	13
Guigne d'Annonay	182	59	32	Big Kentish	148	53	26
Knight's Early Black	366	53	14	Big. Napoleon	258	56	22
Late Black Big.	200	61	30	Black Downton	175	49	28
Monstrueuse de Mezel B	212	53	25	Black Eagle	66	26	39
Mumford Black	219	25	11	Black Heart	145	18	12
Noble	151	21	14	Black Oliver	193	47	24
Noir de Guben	57	12	21	Black Tartarian A	75	34	45
Noir de Schmidt	112	47	42	Black Tartarian B	73	15	20
Peggy Rivers	60	9	15	Black Tartarian E	30	11	37
Preserving	85	24	28	Bohemian Black	46	22	48
Roundel Heart	159	32	20	Cryall's Seedling	99	49	49
Turkey Heart	40	24	60	Early Purple Gean	12	9	75
Ursula Rivers	37	3	8	Early Rivers	454	138	28
Waterloo	218	41	19	Elton Heart	70	10	14
May Duke*	28	11	39	Emperor Francis	362	88	24
Royal Duke*	72	12	17	Florence	22	11	50
Wye Morello*	39	4	10	Géante de Hedelfingen	150	56	37
Prunus decumana	24	13	54	Governor Wood	354	122	34
				Guigne d'Annonay	107	21	20
FLORENCE.				Ham Green Black	100	39	39
Selfed	*188*	*0*	*0*	Knight's Early Black	452	141	31
Bedford Prolific	33	6	18	Late Black Big.	187	66	35
Belle Agathe	110	43	39	Monstrueuse de Mezel B	88	14	16
Big. Jaboulay	36	23	64	Mumford Black	88	37	42
Big. Kentish	66	26	39	Noble	52	20	38
Big. Ludwig's	32	7	22	Noir de Schmidt	116	29	25
Big. Napoleon	67	42	63	Ohio Beauty	41	4	10
Big. Schrecken	53	11	21	Peggy Rivers	131	16	12
Black Heart B	37	15	40	Red Turk	85	26	31
Black Tartarian D	25	3	12	Roundel Heart	113	56	49
Bohemian Black	102	28	27	Smoky Dunn	156	46	29
Emperor Francis	129	40	31	Stark's Gold	551	158	29
Géante de Hedelfingen	99	63	64	Turkey Heart	144	73	51
Governor Wood	62	23	53	Ursula Rivers	26	12	47
Monstrueuse de Mezel B	23	15	65	White Big	140	53	38
Noble	119	55	46	Empress Eugenie*	37	16	43
Noir de Schmidt	53	36	68	Late Duke*	80	0	0
Peggy Rivers	46	27	59	May Duke*	512	72	14
Roundel Heart	27	12	44	Flemish Red*	166	22	13
Stark's Gold	45	24	53	Kentish Red*	23	11	48
Ursula Rivers	20	4	20	Morello*	129	3	2
				Wye Morello*	20	7	35

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
GÉANTE DE HEDELFINGEN.			
Selfed	376	0	0
Hooker's Black	59	0	0
Monstrueuse de Mezel B	214	0	0
Baumann's May	26	6	23
Bedford Prolific	38	16	42
Beeve's Heart	42	4	9
Belle Agathe	6	4	67
Big. Dennison's	29	7	24
Big. Jaboulay	58	35	60
Big. Kentish	113	50	44
Big. Napoleon	262	40	15
Big. Schrecken	129	21	16
Black Circassian	51	11	21
Black Eagle	33	6	18
Black Elton	51	21	41
Black Heart B	85	58	68
Black Oliver	41	30	73
Black Tartarian D	45	11	24
Bohemian Black	18	12	67
Cryall's Seedling	35	4	11
Early Rivers	14	7	50
Elton Heart	110	10	9
Florence	27	9	33
Goodneston Black	52	32	61
Governor Wood	129	9	7
Guigne d'Annonay	29	9	31
Knight's Early Black	93	17	18
Late Amber	86	25	29
Leicester Black	39	3	8
Noble	181	16	9
Noir de Schmidt	177	44	25
Red Turk	93	49	53
Ronald's Heart	72	7	10
Semis de Burr	64	7	16
Smoky Dunn	95	48	50
Stark's Gold	85	18	21
Turkey Heart	65	12	18
Urusla Rivers	197	105	53
Victoria Black	40	11	27
West Midlands Big.	45	7	16
Waterloo	115	32	28
Late Duke*	40	9	22
Flemish Red*	54	9	17
GOODNESTON BLACK.			
Selfed	38	0	0
Late Black Big.	7	6	86
Stark's Gold	37	25	67
GOVERNOR WOOD.			
Selfed	1,711	3	0
Elton Heart	282	0	0
Stark's Gold	228	0	0
Baumann's May	84	24	28
Bedford Prolific	95	37	39
Belle Agathe	169	49	29

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Belle d'Orléans	172	65	38
Big. Jaboulay	69	20	29
Big. Kentish	145	56	39
Big. Napoleon	101	45	44
Big. Schrecken	110	31	28
Black Cluster	118	32	27
Black Eagle	115	18	16
Black Elton	76	43	56
Black Heart B	133	51	38
Black Oliver	79	18	23
Black Tartarian A	24	4	17
Black Tartarian B	123	45	36
Black Tartarian E	24	6	25
Bohemian Black	213	50	23
Early Purple Gean	58	24	41
Early Rivers	300	123	41
Emperor Francis	81	32	39
Florence	43	12	28
Frogmore Early	111	52	47
Géante de Hedelfingen	168	48	28
Goodneston Black	111	21	19
Guigne d'Annonay	45	23	51
Knight's Early Black	32	20	62
Late Black Big.	54	24	44
Monstrueuse de Mezel B	76	14	18
Noble	359	184	51
Noir de Guben	120	45	37
Noir de Schmidt	88	23	26
Peggy Rivers	69	29	42
Red Turk	33	21	64
Roundel Heart	42	30	71
Turkey Heart	123	66	54
Urusla Rivers	38	23	60
Victoria Black	37	28	76
Waterloo	45	22	49
White Big.	244	33	13
Late Duke*	33	6	18
May Duke*	12	4	33
Morello*	24	7	29
Wye Morello*	17	5	29
GUIGNE D'ANNONAY.			
Selfed	669	0	0
Cryall's Seedling	74	0	0
Big. Napoleon	174	100	57
Big. Schrecken	186	68	36
Black Oliver	37	6	16
Early Rivers	186	68	36
Emperor Francis	256	130	51
Goodneston Black	95	56	59
Governor Wood	218	90	41
Leicester Black	38	28	74
Noble	76	52	68
Noir de Schmidt	86	45	52
Stark's Gold	139	56	40
Ursula Rivers	24	13	54
May Duke*	197	74	37

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Kentish Red*	63	13	21
Morello*	105	36	34
Wye Morello*	182	132	72

HAM GREEN BLACK.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	10	0	0
Big. Napoleon	15	3	20
Big. Schrecken	117	92	79
Governor Wood	45	24	53
Knight's Early Black ..	46	22	48

HOOKER'S BLACK.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	64	0	0

KNIGHT'S EARLY BLACK

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	1,244	1	0
Bedford Prolific	379	0	0
Black Eagle	30	1	3
Black Tartarian A ..	120	0	0
Black Tartarian B ..	141	0	0
Early Rivers	235	1	0
Roundel Heart	194	0	0
Belle Agathe	112	10	9
Big. Jaboulay	22	6	27
Big. Napoleon	161	46	28
Big. Schrecken	100	27	27
Black Elton	38	22	58
Black Tartarian E ..	44	12	27
Cryall's Seedling ..	17	6	35
Frogmore Early	99	20	20
Goodneston Black 6 ..	12	2	17
Governor Wood	99	19	19
Guigne d'Annonay	87	28	32
Noir de Guben	47	24	51
May Duke*	116	0	0
Kentish Red*	23	3	13
Morello*	25	9	36
Wye Morello*	15	1	7

LARGE BLACK.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	40	0	0

LATE AMBER.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	82	0	0

LATE BLACK BIGARREAU.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	1,274	2	0
Bohemian Black	517	1	0
Turkey Heart	321	1	0
Bedford Prolific	79	29	37
Belle d'Orléans	18	5	28
Big. Gaucher	87	29	33
Big. Kentish	250	109	44
Big. Ludwigs	174	67	38
Big. Napoleon	106	27	25
Big. Schrecken	317	142	45
Black Downton	102	30	29

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Black Elton	58	13	22
Black Oliver	139	28	20
Early Purple Gean ..	281	60	21
Early Rivers	376	125	33
Emperor Francis ..	127	31	24
Florence	84	26	31
Frogmore Early	8	4	50
Goodneston Black ..	61	14	23
Governor Wood	193	61	32
Knight's Early Black ..	30	12	40
Mumford Black	156	11	7
Noir de Guben	221	37	17
Noir de Schmidt	384	100	26
Preserving	40	16	40
Red Turk	94	40	42
Roundel Heart	143	38	27
Smoky Dunn	85	29	34
Victoria Black	59	12	20
Waterloo	21	5	24
White Big.	105	21	20
Empress Eugenie* ..	75	21	28
May Duke*	65	24	37
Wye Morello*	83	30	36

LEICESTER BLACK.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	199	0	0

MONSTRUEUSE DE MEZEL B.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Selfed	363	0	0
Géante de Hedelfingen ..	206	0	0
Bedford Prolific	61	13	21
Belle Agathe	84	14	17
Belle d'Orléans	50	10	20
Big Kentish	110	28	25
Big. Ludwigs	9	1	11
Big. Napoleon	187	58	31
Big. Schrecken	34	12	35
Black Cluster	40	8	20
Black Eagle	94	9	10
Black Elton	40	14	35
Black Heart B ..	79	41	52
Black Tartarian D ..	153	62	40
Bohemian Black	99	45	45
Elton Heart	17	11	65
Emperor Francis ..	25	4	16
Florence	57	32	56
Frogmore Early	21	12	57
Governor Wood	32	2	6
Guigne d'Annonay ..	57	29	51
Ham Green Black ..	37	16	43
Noir de Schmidt	12	6	50
Peggy Rivers	11	4	36
Stark's Gold	69	36	52
Ursula Rivers	165	46	28
Victoria Black	50	27	54
White Big.	53	10	19
Morello*	35	12	34

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
MUMFORD BLACK.			
Selfed	45	0	0
Big. Ludwigs	19	3	16
Big. Schrecken	27	3	11
Cryall's Seedling	39	10	26
Guigne d'Annonay	30	12	40
Noir de Schmidt	40	3	7
Peggy Rivers	64	7	11
Stark's Gold	51	10	20
NOBLE.			
Selfed	1,275	5	0
Bedford Prolific	150	41	27
Belle Agathe	30	7	23
Big. Jaboulay	114	40	35
Big. Kentish	50	12	24
Big. Napoleon	483	172	36
Big. Schrecken	155	37	24
Black Cluster	21	3	14
Black Eagle	115	14	12
Black Heart	22	10	45
Black Tartarian B ..	25	5	20
Bohemian Black	57	21	37
Cryall's Seedling	41	22	54
Early Rivers	51	20	39
Elton Heart	203	55	27
Emperor Francis	93	14	15
Florence	192	72	37
Frogmore Early	201	73	36
Géante de Hedelfingen ..	96	18	19
Governor Wood	61	10	16
Guigne d'Annonay	40	13	32
Knight's Early Black ..	70	35	50
Monstrueuse de Mezel B ..	211	50	24
Noir de Guben	133	35	26
Noir de Schmidt	101	37	37
Stark's Gold	51	20	39
Turkey Heart	19	12	63
Ursula Rivers	218	75	34
Waterloo	118	38	32
White Big.	495	81	16
Late Duke*	76	17	22
Flemish Red*	123	18	15
Kentish Red*	72	4	5
Wye Morello*	26	2	8
NOIR DE GUBEN.			
Selfed	1,425	2	0
Belle Agathe	122	51	42
Belle d'Orléans	161	74	46
Big. Jaboulay	78	33	42
Big. Napoleon	88	26	29
Big. Schrecken	330	24	7
Black Tartarian E ..	71	33	46
Bohemian Black	288	108	37
Early Purple Gean ..	193	108	56
Early Rivers	160	98	61

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Frogmore Early	36	26	72
Géante de Hedelfingen ..	106	46	43
Governor Wood	62	31	50
Guigne d'Annonay	200	116	58
Ham Green Black	64	33	51
Noir de Schmidt	98	47	48
Roundel Heart	92	34	37
Stark's Gold	59	37	63
May Duke*	67	6	9
Kentish Red*	99	46	46
Morello*	63	21	33
NOIR DE SCHMIDT.			
Selfed	1,617	2	0
Peggy Rivers	209	0	0
Bedford Prolific	67	4	6
Belle d'Orléans	156	27	17
Big. Gaucher	92	29	31
Big. Jaboulay	56	6	11
Big. Kentish	149	28	19
Big. Ludwigs	73	25	34
Big. Napoleon	174	37	21
Big. Schrecken	100	12	12
Black Cluster	42	2	5
Black Eagle	88	12	14
Black Heart B	85	18	21
Black Tartarian A ..	83	2	2
Bohemian Black	234	27	11
Cryall's Seedling	61	30	49
Early Purple Gean ..	42	9	21
Early Rivers	113	11	10
Elton Heart	87	4	5
Emperor Francis	70	10	14
Florence	141	21	15
Frogmore Early	48	22	46
Géante de Hedelfingen ..	17	3	18
Goodneston Black	62	11	18
Governor Wood	31	7	23
Guigne d'Annonay	141	50	35
Knight's Early Black ..	201	47	23
Late Black Big.	615	117	19
Large Black	55	15	27
Monstrueuse de Mezel B ..	62	16	26
Mumford Black	114	38	33
Noble	550	67	12
Noir de Guben	93	19	20
Preserving	30	4	13
Ronald's Heart	112	9	8
Stark's Gold	197	17	9
Ursula Rivers	36	13	36
Waterloo	19	3	16
White Big.	85	8	9
Late Duke*	111	14	13
May Duke*	43	11	25
Royal Duke*	44	10	23
Reine Hortense*	46	9	19
Wye Morello*	31	11	35
Prunus decumana	33	4	12

* Indicates tetraploid varieties.

OHIO BEAUTY.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	*15*	0	0
Big. Napoleon	*24*	0	0
Bedford Prolific	104	7	7

PEGGY RIVERS.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	60	0	0
Noir de Schmidt	30	0	0
Baumann's May	42	13	31
Big. Kentish	65	25	38
Big. Napoleon	67	22	33
Big. Schrecken	22	4	18
Black Downton	27	13	48
Black Elton	28	11	39
Black Oliver	58	12	21
Black Tartarian D ..	242	73	31
Bohemian Black	37	5	13
Early Purple Gean ..	32	6	19
Early Rivers	39	10	26
Goodneston Black	93	61	65
Governor Wood .. .	96	24	25
Knight's Early Black ..	24	13	54
Red Turk	78	27	35

PRESERVING.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	*42*	0	0
Bedford Prolific	23	7	30
Big. Napoleon	50	44	88
Knight's Early Black ..	41	31	76

RED TURK

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	*61*	0	0
Ursula Rivers	*198*	1	0
Black Downton	93	40	43
Black Elton	18	16	89
Governor Wood	89	19	21
Turkey Heart	25	9	36

RONALD'S HEART.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	*103*	0	0

ROUNDEL HEART.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	*52*	0	0
Bedford Prolific	*94*	0	0
Black Eagle	*170*	0	0
Early Rivers	*33*	0	0
Knight's Early Black ..	*67*	0	0
Belle Agathe	36	8	22
Big. Ludwigs	33	3	9
Big. Napoleon	50	21	42
Big. Schrecken	46	9	20
Emperor Francis	92	12	13
Governor Wood	31	27	87
Late Black Big.	71	22	31
Noble	31	22	71
Waterloo	54	26	48

SEMIS DE BURR.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	56	0	0
Big Schrecken	18	0	0
Big Napoleon	42	7	17
Early Rivers	37	4	11

SMOKY DUNN.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	*131*	0	0
Early Rivers	121	31	26
Governor Wood	11	1	9
Red Turk	29	13	45

STARK'S GOLD.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	83	0	0
Governor Wood	*188*	0	0
Bedford Prolific	60	5	8
Big Dennisons	26	17	65
Big. Napoleon	50	27	54
Big. Schrecken	50	9	18
Black Circassian	150	13	9
Black Downton	137	26	19
Black Elton	58	20	34
Black Oliver	75	11	15
Early Rivers	23	15	65
Frogmore Early	27	18	67
Géante de Hedelfingen ..	91	29	32
Goodneston Black	91	12	13
Noble	170	27	16
Noir de Schmidt	130	40	31
Red Turk	112	25	22
Smoky Dunn	48	8	17
Turkey Heart	91	5	5
Ursula Rivers	47	5	11
Victoria Black	93	15	16
Waterloo	119	50	42

TURKEY HEART.

Pollinations.	Flowers pollinated	Fruits matured.	Per cent. matured.
Selfed	*1,319*	3	0
Bohemian Black	*418*	1	0
Late Black Big	*198*	1	0
Bedford Prolific	106	17	16
Belle Agathe	186	20	11
Big Gaucher	66	8	12
Big. Kentish	172	95	55
Big. Ludwigs	178	36	20
Big. Napoleon	191	31	19
Big. Schrecken	177	6	3
Black Circassian	38	3	8
Black Eagle	51	8	16
Black Heart B	89	34	38
Early Rivers	91	8	9
Emperor Francis	130	29	22
Florence	24	7	29
Frogmore Early	172	27	16
Géante de Hedelfingen ..	125	16	13
Governor Wood	95	28	29

* Indicates tetraploid varieties.

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
Guigne d'Annonay	151	34	22
Hooker's Black	38	3	8
Knight's Early Black ..	182	69	38
Monstrueuse de Mezel B ..	232	18	8
Noble	73	18	25
Noir de Schmidt	71	6	8
Ohio Beauty	84	32	38
Roundel Heart	259	15	6
Semis de Burr	65	15	3
Ursula Rivers	36	14	39
Waterloo	202	43	21
White Big.	69	14	20
Yellow Spanish	34	4	12
URSULA RIVERS.			
Selfed	173	0	0
Red Turk	93	0	0
Big. Kentish	111	13	12
Big. Napoleon	88	21	24
Black Elton	60	3	5
Black Oliver	62	15	24
Black Tartarian D ..	169	27	16
Early Rivers	33	5	15
Frogmore Early	106	10	9
Géante de Hedelfingen ..	108	57	53
Goodneston Black	34	5	15
Governor Wood	40	9	22
Guigne d'Annonay ..	102	12	12
Noble	181	55	30
Peggy Rivers	19	6	32
Smoky Dunn	29	2	7
Stark's Gold	173	37	21
Victoria Black	52	7	13
Waterloo	142	20	14
VICTORIA BLACK.			
Selfed	385	0	0
Big. Schrecken	60	0	0
Black Elton	24	0	0
Frogmore Early	63	0	0
Big. Gaucher	15	1	7
Big. Kentish	150	16	11
Big. Napoleon	19	2	10
Black Downton	58	16	27
Géante de Hedelfingen ..	96	7	7
Governor Wood	60	5	8
Knight's Early Black ..	100	22	22
Red Turk	10	3	30

Pollinations.	Flowers pollinated.	Fruits matured.	Per cent. matured.
WATERLOO.			
Selfed	1,086	0	0
Belle Agathe	89	0	0
Big. Schrecken	93	0	0
Black Cluster	65	0	0
Black Heart B	24	0	0
Frogmore Early	113	0	0
Bedford Prolific	204	33	16
Big. Kentish	215	80	37
Big. Napoleon	81	8	10
Black Tartarian A ..	29	10	34
Black Tartarian B ..	215	22	10
Black Tartarian D ..	36	4	11
Early Rivers	94	12	13
Emperor Francis	145	19	13
Florence	78	26	33
Géante de Hedelfingen ..	72	35	49
Knight's Early Black ..	70	6	8
Noble	277	67	24
Noir de Schmidt	75	4	5
Roundel Heart	117	15	13
Ursula Rivers	213	30	14
May Duke*	312	69	22
Morello*	199	94	47
WEST MIDLANDS BIGARREAU.			
Selfed	28	0	0
WHITE BIGARREAU.			
Selfed	231	0	0
Big. Kentish	422	0	0
Big. Ludwigs	85	0	0
Belle Agathe	86	7	8
Big. Jaboulay	96	10	10
Big. Napoleon	329	39	12
Frogmore Early	106	20	19
Prunus decumana	27	3	11
WHITE HEART.			
Big. Schrecken	50	14	28
Early Rivers	205	72	35
May Duke*	200	30	15
Kentish Red*	200	22	11
YELLOW SPANISH.			
Selfed	25	0	0
Early Rivers	31	4	13

* Indicates tetraploid varieties.

FIG. 1.

Bigarreau Noir de Guben. Right hand side and centre, pollinated with Knight's Early Black. Left hand side self-pollinated. 20 days after pollination.

FIG. 2.

Black Tartarian " A " (Group I), showing incompatible pollinations = flowers pollinated 715, fruits set o. Compatible pollinations – flowers pollinated 717, fruits set 74. Average set of fruit from compatible pollinations = 10·3 per cent.

FIG. 3.

Bigarreau Napoleon (Group III), showing incompatible pollinations = flowers pollinated 521, fruits set 0. Compatible pollinations = flowers pollinated 327, fruits set 183. Average set of fruit from compatible pollinations = 55·9 per cent.

FIG. 4

Normal and Albino Seedlings, from Ursula Rivers' crossed Géante de Hedelfingen.

CITRUS MANURING—ITS EFFECT ON CROPPING AND ON THE COMPOSITION AND KEEPING QUALITY OF ORANGES

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THE primary object of the work reported on here was to determine the interaction of the most common artificial fertilizers used in citrus orchards and the influence of such fertilizers on the composition and quality of oranges and on their keeping quality. Preliminary to this, much information was acquired concerning the effect on cropping and on general growth, and as such data should also be interpreted in the light of the later results on composition and quality, it is felt that these results should be presented as the first part of this paper.

TREATMENTS.

An orchard of approximately 2,500 Washington Navel orange trees was selected for this purpose. The trees were all on rough lemon stock and were eight years old in the orchard at the time of applying differential treatments in 1931. The orchard was planted on the quincunx system at the rate of approximately 100 trees per acre, so that it was convenient for the plots to consist of five trees each ; three buffer rows were left between each two plots. Each of the twenty treatments was replicated six times and the plots were laid out by the randomized block method.

The treatments employed were as follows :—

1. Control.
2. $K_3P_3N_3$.
3. $K_2P_3N_3$.
4. $K P_3N_3$.
5. P_3N_3.
6. K_3.
7. $K_3P_2N_3$.
8. $K_3P N_3$.
9. K_3N_3.
10. P_3.
11. $K_3P_3N_2$.
12. K_3P_3N.
13. K_3P_3.
14. N_3.
15. $K_3P_3N_2$ (Nitrogen applied in two equal amounts, one in early spring, before blossoming, and the other just after fruit set, i.e. about six to eight weeks later).
16. $K_3P_3N_3$ (Lime applied at rate of 20 lb. per tree).
17. $K_3P_3N_3$ (Clean cultivation—no weeds or cover crops).
18. K_3P_3 (Leguminous cover crops sown at rate of 10 lb. *Crotalaria juncea* + 10 lb. Iron Cowpeas per acre).
19. K_3P_3N (Leguminous cover crop as for treatment 18).
20. $K_3P_3N_2$ (ditto.).

Citrus Manuring

The symbols used above are to be interpreted as follows :—

K = 1 lb. K_2SO_4 per tree.   P = 2 lb. superphosphate per tree.

K_2 = 2 ,,   ,,   ,,   ,, P_2 = 4 ,,   ,,   ,,   ,,

K_3 = 3 ,,   ,,   ,,   ,, P_3 = 6 ,,   ,,   ,,   ,,

N = 2 lb. $(NH_4)_2SO_4$ per tree.

N_2 = 4 ,,   ,,   ,,   ,,

N_3 = 6 ,,   ,,   ,,   ,,

The fertilizers were broadcast evenly over the whole area allotted to each tree in the orchard in very early spring about a month before blossoming. Approximately four inch irrigations were applied by the basin system immediately after the application of the fertilizers and as nearly as practicable every six weeks thereafter, unless rain replaced irrigation. The lime applied in treatment 16 was thoroughly forked in before irrigation.

SOIL TYPE.

The soil is of mixed origin, mostly colluvial, overlying residual soils. According to mechanical analysis it belongs to the sandy clays, but on account of its well-flocculated condition it behaves more like a loam. Its physical condition ensures good drainage.

The pH varies at different depths and in different plots from 5·5 to 6·5.

In Table I a composite chemical analysis (excluding nitrogen) is given illustrating the general composition of the soil prior to differential treatment.

TABLE I.*

Depth of sample.	Specific resistance at 60°C. ohms.	HCl (S.G. 1·113) Extract.							1% Citric acid extract.	
		CaO %	MgO %	K_2O %	P_2O_5 %	Fe_2O_3 %	Al_2O_3 %	Insoluble Residue. %	K_2O %	P_2O_5 %
0-12 ins. ...	1100	·86	·35	·32	·055	3·35	4·55	87·0	·017	·006
12-24 ins. ...	1250	·68	·34	·32	·051	3·73	5·18	85·7	·017	·005
24-36 ins. ...	1000	·72	·34	·36	·048	4·48	6·60	82·9	·014	·003
36-48 ins. ...	800	·74	·39	·38	·043	4·57	6·71	82·0	·014	·002

* From Kamerman, P. and Klintworth, H.   S. Afr. Dept. Agr. Sci. Bull. 137, 1934.

According to the above Table the soil can be assumed to be well supplied with mineral plant nutrients, especially with " available " K_2O and P_2O_5.

RECORDING AND SAMPLING.

Crop. The fruit from each tree in every plot was harvested, counted and weighed separately. The weighing was conveniently expedited by using a good spring balance suspended from a cross bar resting on the shoulders of two labourers. Attached to the hook of the balance was a large specially constructed pair of tongs which worked on the principle of ice tongs. The two labourers with the weighing arrangement between them walked one on each side of a row of boxes filled with fruit, and by means of the tongs lifted one box after the other ; the recorder read and wrote down the weight of each box.

The trees were stripped of out-season fruit simultaneously with harvesting the ordinary crop, and the number and weight of out-season fruits was recorded separately.
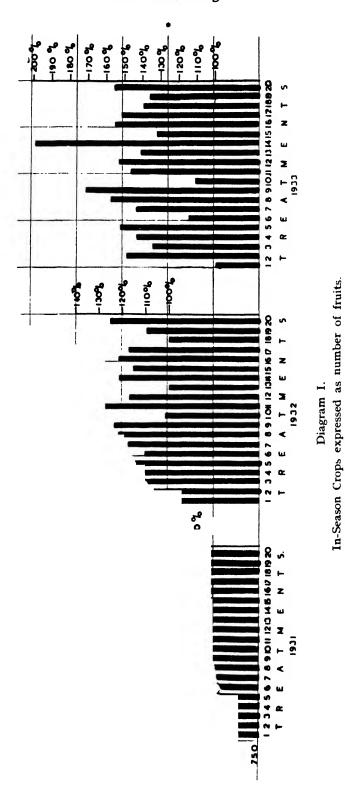
Growth of trees. It has been shown by various investigators (1, 40) that either the cross-sectional area or the circumference of th satisfactory index of the size of the tree. The effect of ferti of tree was accordingly recorded by measuring the circumf of each tree at the time when the treatments were first yearly intervals for the five years of the investigatio first measurement was retained by marking the trunk

Fruit rind thickness. When the fruits wer extracting the juice, the thickness of the rind one point on the cut surface. This figure wa thickness of oranges from all the plots.

Keeping quality. For all records throughout. It was arbitrarily decided of 3⅛ inches, thus packing 126 in the five trees in each plot was grouped order to segregate all the fruits o having been thoroughly mixed, a and packed into a box for storag each representing one of the s boxes. The boxes of oranges w 200 miles to the nearest commer at a temperature ranging fro were kept at atmospheric te period of one to two months for wastage.

No attempt was made fungi, and fruits showing su in cold storage were not cl

Diagram I.

In-Season Crops expressed as number of fruits.

Diagram II.

In-Season Crops expressed as number of fruits.

Chemical analysis. Six fruits, each of a diameter of 3⅛ inches, were taken at random from each tree to make a composite sample of thirty fruits representing each plot.   There were thus six separate samples to be analysed for each of the twenty treatments.   The juice from each sample of thirty fruits was extracted by means of an electric reamer and after thorough mixing aliquot parts were taken for the various analyses.   Samples of rind and pulp were taken just prior to extracting the juice from the same groups of fruit.   The analyses of these will be reported upon later.

Juice content. Representative samples of pulp were procured by cutting a thin cross slice from each orange after it had been halved prior to squeezing. The thirty slices from each sample formed a composite sample.

Meteorological records. A complete meteorological station was established and records taken throughout the period of this investigation.

ANALYTICAL METHODS.

Total soluble solids : By the use of a refractometer graduated to read as percentage sugar.

Acid : Titration with KOH.

Sugar : Picric acid colorimetric method (41).

Juice : The percentage moisture in the pulp was found by drying at 75° C. for forty-eight hours and expressing the difference in weight on the dry basis.

Ash : The percentage ash referred to herein signifies sulphate ash, obtained by igniting with sulphuric acid.

Dry weight of juice : Dried in oven for twenty-four hours at 75° C.

Ca : As calcium oxalate, titrated against $KMnO_4$.

K : As potassium cobalti-nitrite, by Hibbard's method (21).

P : As phosphate, by the molybdate colorimetric method.

N : Kjeldahl method.

STATISTICAL ANALYSIS OF DATA.

All the data collected during this investigation were subjected to statistical analysis according to the methods recommended by Fisher (13), before any conclusions were drawn.   Fisher's Analysis of Variance, " t " test and correlation and regression coefficient calculations having been applied throughout, it merely remains, when presenting the results in the following paragraphs, to indicate whether the results, if significant, are so at the 5% (represented in the Tables, *) or 1% point (represented, **).

RESULTS AND CONCLUSIONS.

Number of fruits. Diagrams I and II represent the effect of treatment on the number of the in-season crop. The mean number of fruits per plot per treatment is determined by reference to the figures on the left of the diagrams. The figures alongside the vertical line on the right of each diagram express the mean for each treatment as a percentage of the control (treatment 1), and the control is thus always indicated as 100%. Furthermore, "significance lines" are shown running across the diagrams which show at a glance when increases or decreases in crop over control are significant at the 5% point or at the 1% point. It will be noted that most of the plots yielded a crop less than the control plots during the pre-treatment year, namely 1931. Although the variability in yield was so great that no significant difference in number of fruits due to treatment can be proved during the first two years after treatment— 1932 and 1933—it is very striking that during both those years, nearly all treatments induced crops greater than that of the control. Furthermore, these increases over the control were progressive from year to year.
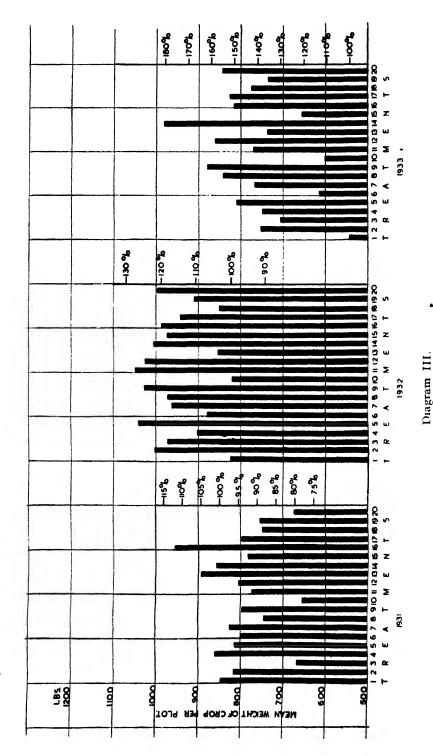
It is only in 1934, 1935 and 1936 that it is possible to prove statistically an increase in the number of fruits. The upper solid horizontal line cutting the vertical bars represents significance at the 1% point and the lower broken line significance at the 5% point. It should be made clear that the distance from a significance line to the top of the bar representing the control, can be used also as a criterion of significance between any other two treatments.

The outstanding results of these treatments is the marked effect of applications of nitrogen in increasing the number of fruits. All plots receiving nitrogen —irrespective of amount—yielded a significantly increased crop, whereas treatments 6, 10 and 13 (K_1, P_3 and K_3P_3) induced crops which may be considered within the range of the experimental error from the control plots.
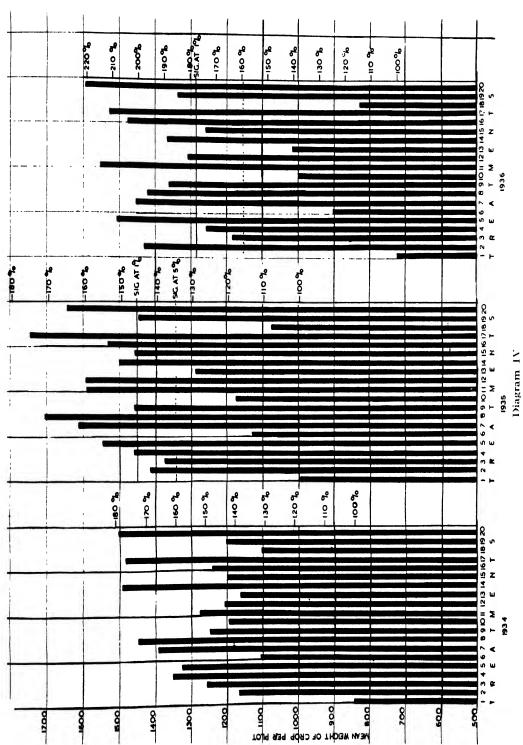
Furthermore, there is no demonstrable difference resulting from the various quantities of nitrogen applied, so that it may be assumed that as far as number of fruits is concerned in this orchard, no particular benefit can be expected from applying more than 2 lb. of ammonium sulphate per tree.

The application of nitrogen in two doses (treatment 15) has not been reflected in an increased crop over treatment 11 when the same total amount of nitrogen was applied at one time, nor has the application of lime resulted in an increased crop. Clean cultivation (treatment 17) also shows no change in number of fruits over treatment 2 where a volunteer weed cover crop was allowed to grow ; the duration of the investigation presumably did not allow any possible effect to manifest itself, although the lack of application of organic matter has been known to manifest itself in three years (31).

The last three treatments, which include an annual leguminous cover crop, again raise the contentious question as to whether a leguminous cover crop

Diagram III.

In-Season Crops, expressed as weight of fruits.

Diagram IV

In-Season Crops, expressed as weight of fruits.

always increases the nitrogen content of the soil. Treatment 18, in which a leguminous cover crop was grown but no nitrogen was added as a fertilizer, returned very poor crops indeed, showing no demonstrable increase or decrease over the control. The significant increase in number of fruits from treatments 19 and 20 over treatment 18 may be attributed to the nitrogen added as fertilizer and not to the leguminous cover crop. At any rate there is no significant difference between the crops from treatments 20 and 11 which are identical except that in the former a leguminous cover crop was grown.

There appears to be no consistent increase from year to year in the number of fruits from the control plots. Although measurements have shown a definite increase in the size of trees from all plots, including the controls, the control trees have not produced a progressively larger crop. It is assumed, therefore, that nitrogen is the limiting factor in this orchard, so that although the trees became larger every year, the limited nitrogen supply induced approximately the same crop. The generally small crops in 1932 and 1933 can be put down to the fact that the trees suffered to a certain extent from drought conditions during those years, when irrigation water supplies were depleted.

The very significant increase during 1935 and 1936 in numbers of fruits in plots where nitrogen was applied cannot be ascribed to a greater increase in size of these trees than in the controls, for it will be shown later that trees receiving nitrogen had not increased in size over trees in the control plots.

Weight of crop. Diagrams III and IV illustrate the results for the weight of crop in the same manner as I and II present the data for the number of fruits. It will be noted that these diagrams follow very closely those for number of fruits, so that the same conclusions hold for both. There is merely one important difference, namely, that no significant increase in weight due to nitrogen treatment can be proved statistically for 1934, whereas the number of fruits in 1934 was increased significantly at the 5% point by nitrogen treatment.

Size of fruits. Actual sizes of fruits were not recorded during this investigation, but an estimate of size was determined by adjusting (by the analysis of co-variance) weight for number, two factors which showed a very high positive correlation annually. The analysis of co-variance did not, however, show any significant effect due to treatment, so that it can be concluded that size (i.e. weight per orange) is approximately the same for all treatments. This, then, confirms the conclusion to be drawn from the close similarity between the diagrams, namely that any increase in weight of crop is due to an increase in number of fruits and not to an increase in size of the fruits.

Out-season fruits. The numbers of out-season fruits harvested are represented in diagrams V and VI in the same manner as the data for in-season fruits. In order to save space in reproduction, the weights of out-season fruits

are not shown here, but such diagrams closely follow V and VI, in the same way that I and II and III and IV follow much the same tendencies.
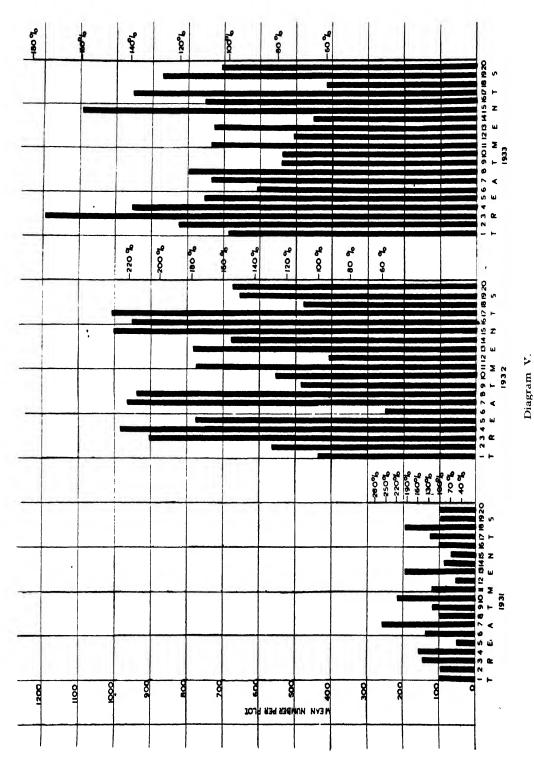
As indicated above, the number and weight of out-season fruits were recorded in the same manner as for the normal seasonal fruits. The main object of this was to keep a record of all the fruit borne by each tree and thus to have an approximate estimate of the drain on the nutritional supplies of the tree. It is popularly contended by growers that a tree bearing a heavy out-season crop will bear a poor in-season crop the following year. From a nutritional point of view this seems a reasonable assumption provided that nutrition is a limiting factor ; and it was one of the objects of this investigation to acquire some information on this point and on the influence of fertilizers on the size of the out-season crop.

On determining an analysis of variance for number of out-season fruits, it was found that during no year was the number of out-season fruits significantly affected by treatment.
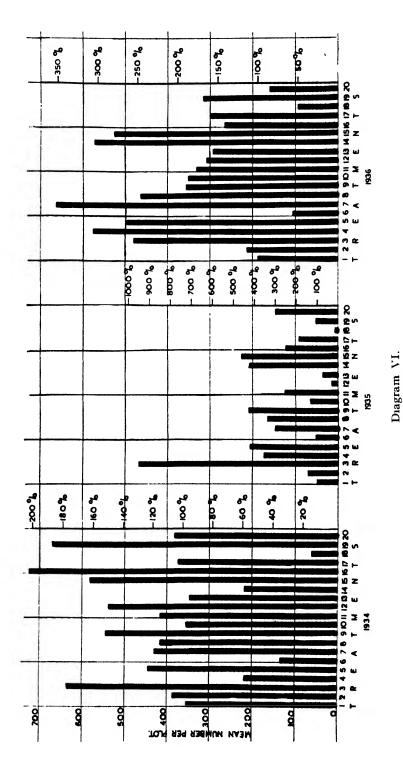
Any possible differential effect of fertilizers on number of out-season fruits was completely masked by apparently more important factors, such as moisture and temperature relationships.

As treatment did not appear to affect the number of out-season fruits it was decided to determine whether the number of in-season fruits was correlated with that of out-season fruits during any one year. The calculation of correlation coefficients showed a very strong negative correlation, significant at 1%, during 1931, 1932, 1933 and 1934 ; during 1935 there was also a negative correlation but not a significant one, and in 1936 a negative correlation significant at 5%. This is what might be expected from a nutritional point of view when account is taken of the tremendous drain on the nitrogen supply of the tree occasioned by blossoming and setting of the fruit (7) and by allowing the fruit to hang on the tree (8). When a large crop of in-season fruit sets during spring, the nitrogen and possibly other nutrient supplies of the tree are so depleted that any blossoming which may take place during the following summer . would not cause much fruit to be set ; thus, it is found that a large in-season crop is correlated with a small out-season crop and vice versa. The fact that this negative correlation was not as significant during 1935 and 1936 as during the first three years, is explained by the accumulation of nitrogen that took place during the first three years of the investigation, due to treatment.
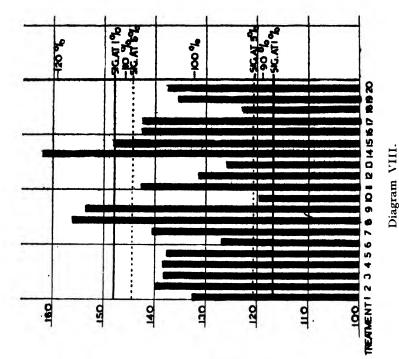
The next test applied to the data was to determine whether a large out-season crop in one year would cause a small in-season crop during the following year. With the exception of the 1932 out-season and the 1933 in-season crop, none of these calculations for the five years of the investigation showed any significant correlations whatsoever. The negative correlation, significant at 1%, between the 1932 out-season and the 1933 in-season crop might possibly
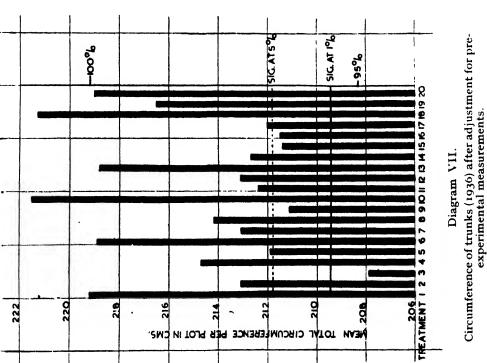
Diagram V.

Out-Season Crops, expressed as number of fruits.

Diagram VI.

Out-Season Crops, expressed as number of fruits.

Diagram VIII.
Thickness of rind (1936), in mm.

Diagram VII.
Circumference of trunks (1936) after adjustment for pre-experimental measurements.

be explained by the fact, shown in diagrams V and VI, that during 1932 there was a heavy out-season crop ; this may have been brought about by irregular moisture conditions during this year, when the country experienced a very severe drought, or by the fact that the trees were low in nitrogen at the start of these investigations and had not yet accumulated sufficient nitrogen from the first application of fertilizers to set a heavy in-season crop (diagram I, 1932), but could, during the following summer, set a heavy out-season crop.

This nearly general lack of correlation means that as the out-season fruit was harvested simultaneously with the in-season fruit (early winter) the trees had sufficient time to build up enough reserves of food materials before spring and blossoming time to set a good crop of in-season fruit, irrespective of the size of the previous out-season crop. Had the out-season fruit, however, been allowed to mature on the tree, then it is probable that the trees would have been unable to set a good crop of in-season fruit during the following spring ; in such a case there would most probably be a significant negative correlation between one year's out-season crop and the following year's in-season crop. This assumes, however, that only enough nutrient materials are available to the tree to bear one crop per year ; it is conceivable, on the other hand, that the constant availability of large quantities of food materials may make it possible for a tree to mature a considerable out-season crop as well as a good in-season crop.

In order to determine whether the procedure of pulling off the green out-season fruit during the harvesting period of the in-season fruit had any effect on the "habit" of trees to bear out-season fruit, correlations were calculated between the number of out-season fruits from year to year. A positive correlation, significant at 1%, was found between the numbers of out-season fruits for 1931-2, 1932-3, 1935-6 and 1931-6, but no significant correlation between those of 1933-4 and 1934-5. It would be legitimate to conclude from this that, contrary to the commonly expressed opinion of growers, the stripping of trees of out-season crops at the time of harvesting the normal crop does not influence the so-called "habit" of trees to bear out-season fruit. As has been suggested above, however, this stripping of the trees of out-season fruit may affect the following in-season crop favourably if the trees have not a considerable nutritional reserve.

Growth of trees. The appearance of the trees from the point of view of colour and density of foliage and of general vigour and size showed no obvious differences due to treatment.

An indication of size of tree as affected by treatment was procured by analysing statistically the data for the circumferences of trunks of trees. An analysis of variance of the data showed no significant difference in the circumferences due to treatment during any of the five years. However, when
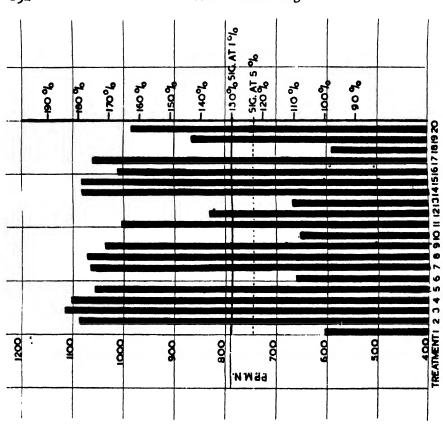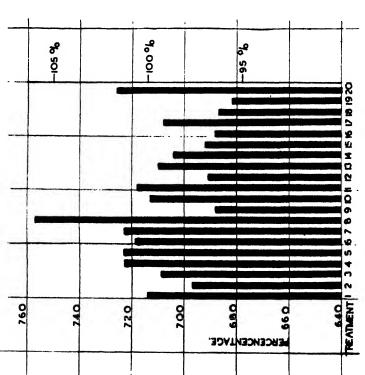
Diagram X.

Total Nitrogen in juice (1936).

Diagram IX.

Moisture in 1936 pulp, expressed on dry weight basis.

the circumference data were adjusted for 1931, the pre-treatment year, then differences due to treatment were very definitely significant. In 1933 the treatment effect was significant at 5%, and in all the following years it was significant at 1%. The data for the various years are very similar, therefore only those for 1936, adjusted for 1931, are included here and represented in diagram VII. The rather surprising conclusion to be drawn from this diagram is that it is the control and the treatments without nitrogen that have shown the most marked increase in circumference, and presumably in growth generally. Treatments 1, 6, 10, 13 and 18, none of which included nitrogen, have thus resulted in the greatest increase in tree size. Treatments 19 and 20, which do include nitrogen, also show increases in circumference above the rest of the treatments, but these two cases, in which there was a luxuriant growth of the cover crop, may have been influenced by that crop in some obscure way ; at any rate no explanation can be offered at this juncture.

It will be noted that the differences in circumference illustrated in diagram VII are quite small, owing to the adjustment of the data for differences existing in the pre-treatment period, so that the actual differences due to treatment, although significant in a few cases, are unimportant from a practical point of view. Presumably, such differences as do exist with treatments not receiving nitrogen could be ascribed to the fact that such trees set only very small crops, and that the bulk of the relatively small amount of nitrogen present was available for growth. This, again, is an illustration of the tremendous drain which the setting and development of a crop exercise on the nitrogen reserves of a tree.

Rind thickness. The thickness of rind was determined only during 1936, the final year of this investigation, by the method described in an earlier paragraph. The analysis of variance of the data showed treatment to affect the thickness of rind significantly at 1%. The results are shown in diagram VIII.

Although relatively few of the vertical bars cross the significance lines in this diagram, there are two general conclusions which can be drawn with a fair degree of accuracy. Treatments 6, 10, 12, 13 and 18 are the only ones in which the average thickness of rind is less than that of the control and, including the control itself, these six treatments show thinner rinds than any of the other treatments. These six treatments are also the only ones in which no nitrogen was given (except treatment 12, in which only a very small amount of nitrogen was added), and it will be shown later that, for this reason, the trees have absorbed most phosphate. The six treatments resulting in the thinnest rind have the highest phosphate content, so that the indication is very strong that a high phosphate content results in a thin rind.

Diagram XII.

Calcium in juice (1936).
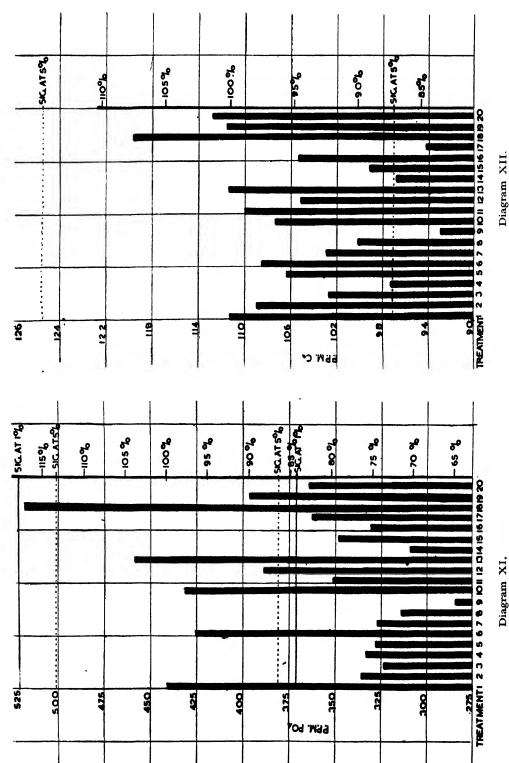
Diagram XI.

Total Phosphorus in the 1936 juice, expressed as PO₄.

The remaining treatments all include a dressing of nitrogen, and in the majority of them the heavy application of six pounds of ammonium sulphate per tree. Furthermore, the three treatments 8, 9 and 14 which induced the most significantly thick rinds are those in which heavy applications of nitrogen (N_3) and little or no phosphate were given. The fruit from these plots will be shown later also to have absorbed the smallest amounts of phosphate.

The general conclusion indicated here is that low phosphate and high nitrogen is associated with thick rind and vice versa, but it will be shown later, where partial regression coefficients have been calculated, that the nitrogen effect is merely an indirect one, due to its depressing effect on absorption of phosphate, and that it is really the latter only that has a direct effect on the thickness of rind.

Juice content. As indicated earlier in this paper, the juice content was not determined in the orthodox fashion of calculating the percentage of juice by weight when rind is included ; this method was not used because any change in thickness of rind would alter the calculated juice percentage of the whole, whereas actually there may have been no change in the juice content of the pulp itself.

The data have been treated statistically throughout, but at no time have any of the treatments significantly affected the percentage moisture in the pulp. The results are presented in diagram IX.

In the literature (1, 35) it is mentioned that applications of nitrogen decrease the juice content of oranges, and that phosphate applications have the reverse effect. It should be kept clearly in mind that in the present paper it is the juice in the pulp only that has been determined, and although there were large fluctuations in nitrogen and phosphate content according to treatment, there was no effect on the moisture content of the pulp. This seeming contradiction will be explained later when partial regression coefficients are discussed, and when it will be shown that nitrogen depresses the absorption of phosphate and that a low phosphate content causes a thick rind. Because of these relationships, it will be clear why nitrogen and phosphate would affect the juice content when it is calculated on a whole fruit basis including rind.

Nitrogen content of juice. The nitrogen content of the juice was determined and the results for 1936 are presented in diagram X.

All nitrogen applications have significantly increased the nitrogen content of the juice. There is furthermore a significant difference between the nitrogen content of juice from plots receiving a light application of nitrogen (treatments 12 and 19) and those receiving a heavy application.

Reference was made earlier to the contentious question as to whether a leguminous cover crop appreciably increases the nitrogen content of all soils.

Diagram XIV.
Sulphate Ash weight of 100 cc. juice (1936).

Diagram XIII.
Potassium in juice (1936).
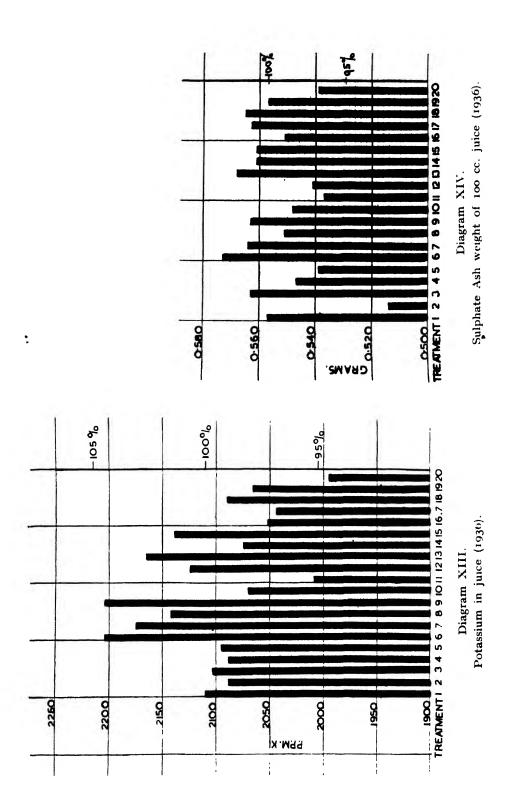
Diagram X again raises this question. Treatment 18 is obviously no better off for nitrogen than the control plots or the others that have not received nitrogen ; also treatment 19, which provided for only a small amount of nitrogen and a leguminous cover crop, shows no higher nitrogen content of the juice than does treatment 12 in which no leguminous cover crop was included.

The exceedingly close relationship between the nitrogen content and treatment confirms the statements made earlier concerning the effect of treatment on number and weight of fruits.
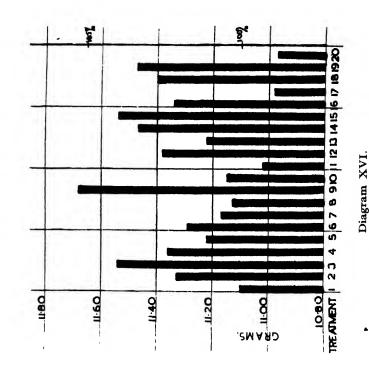
Phosphorus content of juice. The samples of juice were analysed first for inorganic phosphorus and a second aliquot part was then ignited with magnesium nitrate and the total phosphorus determined. The organic phosphorus was calculated by difference. Only total phosphorus is presented in diagram XI in order to show the amount of phosphorus absorbed from the soil. The relationship between the inorganic and organic forms of phosphorus will be considered in a later section.

It will be noted that diagram XI shows exactly the reverse of the nitrogen figures presented in diagram X. Wherever nitrogen has been omitted (treatments 1, 6, 10, 13 and 18) or applied in small quantities only (treatments .2 and 19), the phosphorus content of the juice is high—higher in the former than in the latter. All treatments involving medium or high applications of nitrogen result in fruit juice with a phosphorus content very significantly below that from the rest of the plots.

This very significant relationship between nitrogen and phosphorus will be discussed further later in this paper when the various correlations are considered.

Calcium content of juice. The concentration of calcium in the juice for the 1936 season is presented in diagram XII. Although at first glance there appear to be large fluctuations in calcium content from treatment to treatment, it will be noticed from the figures on the left of the diagram that the concentrations in all the treatments vary only from 93 to about 120 p.p.m. Ca. There is no significance line for the 1% point because none of the fluctuations due to treatment is significant to this extent. Treatments 9, 14 and 17 show calcium contents below that of the control, the differences being significant at 5%. These low concentrations do not appear to be correlated with the particular treatments, however, and no explanation is offered at this stage. The concentrations are presented here, because the question of calcium content will be discussed again in a later paragraph dealing with correlations. In passing, however, attention should be drawn to treatment 16 which included annual applications of lime at the rate of 20 lb. $CaCO_3$ per tree ; these plots do not show any response in the calcium content of the fruit juice.

Potassium content of juice. The potassium concentration of juice in 1936 is illustrated by diagram XIII. As with calcium, the fluctuations in potassium

Diagram XVI.
Dry weight of 100 cc. juice (1936).
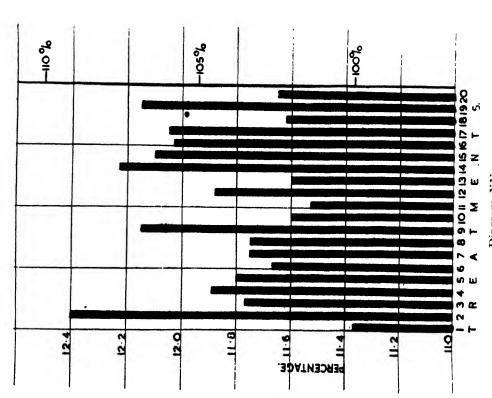
Diagram XV.
Total Soluble Solids in juice (1936).

content are relatively narrow and there are no significant differences due to treatment. It can be assumed, however, that very little of the potassium applied with the treatments was available for absorption in the root zone because of the rapid fixation of potassium in the upper layer of soil.

The data presented here will be considered again later in the paragraphs concerned with correlations.
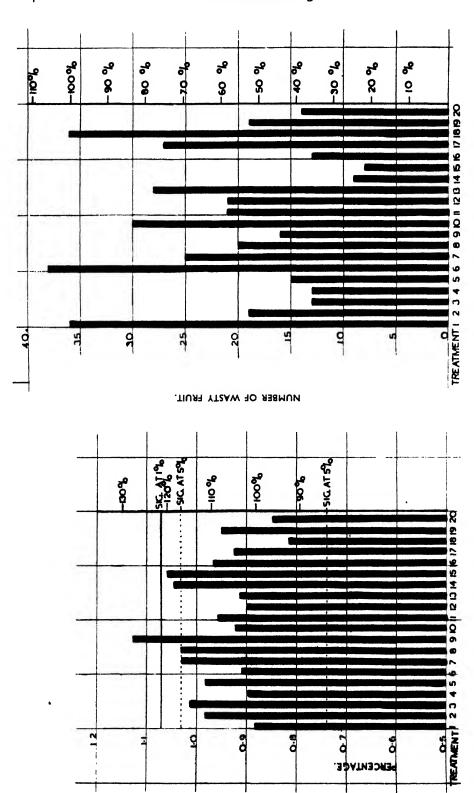
Ash weight of juice. The ash weight of juice was determined as indicated at the outset and the results are presented in diagram XIV. There are no significant differences in ash content due to differential treatment, but the data will be referred to again in the later paragraphs dealing with correlations and partial regression coefficients.

Sugar content of juice. The juice was analysed for total sugars, but as the statistical analysis of the data produced no significant differences due to treatment, it is felt that they need not be reproduced here. The approximate quantities of sugars present in the juice can be gauged by the refractometer readings referred to in the next paragraph, which represent total soluble solids of which sugars form the major part. Further consideration will be given to the data later in the paragraphs dealing with correlations.

Total soluble solids in juice. The total soluble solids as determined by a refractometer are represented for 1936 in diagram XV. It will be noted that there are no significance lines across this diagram, thus showing that the differential treatments had no significant effect on the soluble solids content of the juice. In view of the lack of significance with sugar content and also with ash content, it might be expected that total soluble solids also would not show any significant differences due to treatment. These data also will be referred to again when discussing correlations in a later paragraph.

Dry weight of juice. The dry weight of juice reflects much the same picture as the concentrations of total soluble solids determined with the refractometer. The results are presented in diagram XVI and will be discussed again later when the various correlations are considered. It will suffice at this stage to point out that treatment had no significant effect on the dry weight of juice.

Acid content of juice. It will be noted from diagram XVII that very few of the vertical bars representing acid content of juice cross the 5% significance line and that only one crosses the 1% significance line. However, it appeared from this diagram as if the treatments with nitrogen showed a generally higher acid content than the rest, hence an analysis of variance was calculated, grouping together all the treatments receiving nitrogen (2, 3, 4, 5, 7, 8, 9, 11, 12, 14, 15, 16, 17, 19 and 20) and all those without nitrogen (1, 6, 10, 13 and 18). As a result of this analysis the " Z " value for nitrogen treatments versus the

Diagram XVIII.

Total number of waste fruit per treatment in packed cases (1936)

Diagram XVII.

Acid content of 1936 juice, expressed as Citric acid.

rest was markedly significant at 1%. It can accordingly safely be concluded that nitrogen applications to these trees caused the fruit to have a higher acid content. The question as to whether this effect is a direct or an indirect one will receive consideration in the later paragraphs dealing with correlations.

Wastage during storage. Diagram XVIII represents the 1936 results of wastage counts in six cases of oranges from each treatment. It will be noted that no horizontal significance line is indicated because the analysis of variance of the data showed an insignificant " Z " value. This diagram shows rather strikingly, however, that the five treatments (1, 6, 10, 13 and 18) which had not included nitrogen, are also the five which show the greatest amount of wastage. For this reason an analysis of variance was calculated, when all the nitrogen treatments were grouped together and compared with the non-nitrogen treatments. In this analysis the " Z " value was significant at 1% ; in other words, the nitrogen treatments significantly decreased the amount of wastage in the fruit. This point also will be considered further in the following paragraphs dealing with correlations.

CORRELATION AND REGRESSION COEFFICIENTS.

In the preceding paragraphs the results of differential manurial treatments on the crop, composition and other characters of oranges have been reported. The data so acquired have been further analysed with a view to determining what correlations, if any, exist between the various characteristics. Table II gives the results of such correlation coefficients, which are to be interpreted as correlations which exist *irrespective* of differential treatments. Usually only those correlations significant at 1% will be considered in the following paragraphs.

The units used in the partial regression coefficients given below, are the same as those used in the previous diagrams.

ACID CONTENT.

Acid × Moisture. Coefficient No. 1 shows a negative correlation, significant at 1%, between acid content of juice and moisture content of pulp. This presumably means merely that when the juice content of an orange is high its acid content is relatively low due to dilution.

Acid × Thickness of rind. Coefficient No. 16 shows that the acid content is significantly and positively correlated at 1% with the thickness of the rind. As these two factors cannot very well be directly correlated, the explanation for the correlation must lie in some third factor with which both of these characters are strongly correlated. Coefficients 20 and 33 give the necessary explanation. Here phosphorus content is shown to be negatively and significantly correlated at 1%, first with acid content and then with thickness of rind.

TABLE II.

Correlation Coefficients of Juice Components, Juice Content (Moisture) of Pulp, Thickness of Rind and Wastage.

	Acid.	Thickness of rind.	Wastage.	Total Soluble Solids.	Total Phosphorus.	Inorganic Phosphorus.	Organic Phosphorus	Sugar.	Nitrogen.	Potassium.	Calcium.	Ash.	Dry Weight.
Moisture content of pulp	(1) −0·416**	(2) −0·165	(3) 0·297**	(4) −0·605**	(6) 0·110	(7) 0·082	(8) 0·054	(9) −0·499**	(10) −0·367**	(11) −0·349**	(12) 0·292**	(13) −0·346**	(15) −0·539**
Acid		(16) 0·587**	(17) −0·458**	(18) 0·555**	(20) −0·541**	(21) −0·547**	(22) 0·049	(23) 0·247*	(24) 0·581**	(25) 0·363**	(26) −0·514**	(28) 0·227*	(29) 0·457**
Thickness of rind			(30)	(31)	(33)	(34)	(35)	(36)	(37)	(38)	(39)	(41)	(42)
Wastage				(43) −0·440**	(45) 0·348**	(46) 0·401**	(47) −0·140	(48) −0·222*	(49) −0·530**	(50) −0·252*	(51) 0·568**	(53) −0·188	(54) −0·401**
Total Soluble Solids					(56) −0·133	(57) 0·185	(58) 0·124	(59) 0·705**	(60) 0·561**	(61) 0·507**	(62) −0·443**	(64) 0·473**	(65) 0·830**
Total Phosphorus						(76) 0·895**	(77) 0·165	(78) −0·047	(79) −0·385**	(80) 0·147	(81) 0·294**	(83) 0·207*	(84) 0·170
Inorganic Phosphorus							(85) −0·292**	(86) −0·121	(87) −0·494**	(88) 0·103	(89) −0·345**	(91) 0·107	(92) −0·249*
Organic Phosphorus								(93) 0·169	(94) 0·267**	(95) 0·086	(96) −0·134	(98) 0·208*	(99) 0·187
Sugar									(100) 0·344**	(101) 0·339**	(102) −0·159	(104) 0·436**	(105) 0·797**
Nitrogen										(106) 0·399**	(107) −0·474**	(109) 0·224*	(110) 0·512**
Potassium											(111) −0·306**	(113) 0·641**	(114) 0·489**
Calcium												(116) −0·231	(117) −0·285**

* 5% significance.   ** 1% significance.

It therefore seems justifiable to conclude that the correlation between acid content and thickness of rind is an indirect one.

Acid × Wastage. Coefficient No. 17 shows a very strong negative correlation, significant at 1%, between acid content and wastage. This may possibly be a direct correlation, as it is conceivable that the higher the acid content the less favourable the medium may become for fungus growth, hence less wastage. It is possible, however, that the acid content may be associated with wastage only indirectly, in the following manner: Acid content is negatively correlated with calcium content (coefficient No. 26) which, in turn, is positively correlated with wastage (coefficient No. 51). As a matter of fact it will be shown later, under the heading, "Wastage", where partial regression coefficients have been calculated for all the factors correlated with wastage, that this relationship between acid content and wastage is indirect.

Acid × Total soluble solids. The significant positive correlation between these two factors (coefficient No. 18) might have been expected, because the acid present is always one of the components included in the determination of total soluble solids.

Acid × Total P, N, K, Ca and Dry weight. Acid content is correlated negatively with total phosphorus (No. 20), positively with nitrogen (No. 24) and potassium (No. 25), negatively with calcium (No. 26) and positively with dry weight (No. 29). As there is insufficient actual information available concerning the physiological influence of these factors to make it possible to interpret the above correlations with certainty, partial regression coefficients of acid content and total phosphorus, nitrogen, potassium, calcium and dry weight were calculated.

From Fisher's "t" table it was determined that for any of these partial regression coefficients to be significant, their "t" values should exceed 1·987 at the 5% point and 2·634 at the 1% point.

The partial regression coefficients and "t" values of the above factors are the following:—

	Partial Regression Coefficients.	"t" Values.
Acid and P	− 0·001,039 ± 0·000,195	5·322**
,, ,, N	0·000,163 ± 0·000,097	1·675
,, ,, K	0·000,251 ± 0·000,092	2·717**
,, ,, Ca	− 0·002,034 ± 0·000,811	2·510*
,, ,, Dry weight	0·024,154 ± 0·016,269	1·485

It will be noted that, of the five factors tested, an influence significant at 1% is exerted only by the phosphorus content (negatively) and by the potassium content (positively), although the effect of calcium is also significant at 5%.

From the above it is to be concluded that although nitrogen content is consistently positively correlated with acid content, and the nitrogen applications reported on in earlier paragraphs are also associated with increased acid content, the high acid content of the fruit is not directly attributable to high nitrogen content. Rather, it is the low phosphorus content (which will be shown by later partial correlations to be induced by high nitrogen) which is the cause of the increased acid content. It is thus conceivable that oranges may have a high nitrogen content and still exhibit no more than a normal acid content, provided that the phosphorus content of the fruit is also high.

The potassium content is positively correlated with acid content, and from the above calculation of partial regression coefficients it is evident that potassium has a direct influence on acid content and is not merely indirectly correlated with it.

Calcium is associated with acid content and in a negative manner, but this influence is significant only at the 5% point. However, the same effect of calcium is reported by other workers (18), so that this significance at 5% can most probably be accepted as presenting a true influence of calcium on acid content.

THICKNESS OF RIND.

Rind × Total soluble solids. Coefficient No. 31 shows a positive correlation, significant at 1%, between these two factors. As it is not easily conceivable how a high concentration of total soluble solids could induce a thick rind, this correlation must for the time being be considered an indirect one.

Rind × Total P, N, K, Ca and Dry weight. The thickness of rind is negatively correlated with total phosphorus (No. 33) and positively with nitrogen (No. 37), potassium (No. 38) and with dry weight (No. 42).

A calculation of partial regression coefficients of thickness of rind and the above factors gave the following results, where Fisher's " t " table requires a value of 2·634 for significance at 1% and of 1·987 at 5%.

					Partial Regression Coefficients.	" t " Values.
Thickness	of	rind	and	P	−0·087,381 ± 0·020,132	4·341**
,,	,,	,,	,,	N	0·012,486 ± 0·010,047	1·240
,,	,,	,,	,,	K	0·031,278 ± 0·009,527	3·283**
,,	,,	,,	,,	Ca	0·066,342 ± 0·083,537	0·794
,,	,,	,,	,,	Dry weight	0·574,476 ± 1·676,59	0·343

Of the four factors it appears that only phosphorus content and potassium content have a direct effect on thickness of rind, the former being negatively and the latter positively associated.

It is the practical citrus grower's experience generally that heavy applications of nitrogenous fertilizers cause the fruits to have thick rinds. From the above it would appear that the nitrogen effect is an indirect one and that its influence in retarding absorption of phosphorus (which will be further proved later) is the real reason for the thick rind. It would be reasonable to assume from the above that a high nitrogen content in the soil or fruit would not induce thick rinds provided that large quantities of phosphorus were also available and absorbed. The remarks concerning the relationship of nitrogen and phosphorus made in the previous paragraphs dealing with acid content would also apply here.

The lack of significance for dry weight in the above Table confirms the opinion expressed in the preceding paragraph, namely that the correlation of thickness of rind with total soluble solids (in this case, dry weight) is merely an indirect one.

Wastage.

From Table II it will be seen that the following factors are correlated, at 1%, with wastage: Moisture content of pulp (+), acid (−), thickness of rind (−), total soluble solids (−), total phosphorus (+), nitrogen (−), potassium (−, actually only at 5%, but included here because of its alleged importance in connection with storage quality), calcium (+) and dry weight (−).

Because it was impossible to assess the importance of the above factors separately and to determine which correlations were direct or indirect, the tremendous task of solving partial regression coefficients of wastage and the above nine variables was decided upon. The coefficients for the nine factors were as follows when Fisher's " t " table requires their " t " values to exceed 1·987 for significance at 5% and 2·634 at 1%.

	Partial Regression Coefficients.	" t " Values.
Wastage and moisture in pulp	0·000,849 ± 0·007,3	0·116
,, ,, acid	− 0·499,989 ± 4·467,9	0·112
,, ,, thickness of rind	− 0·004,305 ± 0·414,0	0·010
,, ,, total soluble solids	− 0·010,144 ± 1·094,6	0·009
,, ,, Total P	0·009,124 ± 0·008,9	1·025
,, ,, N	− 0·007,533 ± 0·003,7	1·998*
,, ,, K	− 0·000,694 ± 0·003,7	0·186
,, ,, Ca	0·125,089 ± 0·033,0	3·663**
,, ,, Dry weight	− 0·969,337 ± 0·931,2	1·041

The only factor which at 1% significantly influences wastage in these oranges was the calcium content. The more calcium present in the fruit the more serious was the wastage due to mould growth.

The value for nitrogen in the above Table is significant only at 5% and should accordingly be accepted with reserve. By referring forward to the paragraph dealing with the effect of treatment on wastage, however, it will be noted that " N-treatments versus the Rest " gave a " Z " value significant at 1%, so that combined with the above partial regression coefficient for wastage and nitrogen, significant at 5%, the conclusion that high nitrogen induces low wastage is more justified.

<div align="center">SUGAR CONTENT.</div>

Sugar × Moisture content of pulp. Coefficient No. 9 in Table II shows a negative correlation, significant at 1%, between sugar content of juice and moisture content of pulp. It is assumed that this correlation is to be attributed to the dilution effect of an increased moisture content of the pulp on the concentration of the sugar.

Sugar × Total soluble solids. This correlation (No. 59) is to be expected when it is considered that sugar is the principal constituent of the total soluble solids. The same remarks hold for coefficients Nos. 105 and 65 where sugar and total soluble solids are very highly correlated with dry weight.

Sugar × N, K and Ash. Sugar content is significantly and positively correlated with nitrogen (coefficient No. 100), potassium (No. 101) and with ash (No. 104). In order to determine which of these three factors directly affect sugar content, partial regression coefficients were calculated of sugar content and nitrogen, potassium and ash content. The " t " values of these regression coefficients must exceed 2·634 to be significant at 1%, and 1·987 at 5%. They are shown in the following Table.

	Partial Regression Coefficients.	*" t " Values.*
Sugar and N	0·001,256 ± 0·000,467	2·692**
,, ,, K	−0·000,064 ± 0·000,602	0·106
,, ,, Ash	8·190,350 ± 2·494,180	3·284**

The above Table shows that only nitrogen content and ash content influence sugar content, potassium does not affect sugar content directly. The apparent affect of potassium on sugar content, as indicated by coefficient No. 101, must be assumed to be an indirect one, brought about by the positive correlation between potassium and nitrogen (No. 106) and between nitrogen and sugar (No. 100).

The above regression coefficients mean, furthermore, that there is some constituent or constituents in the ash other than N or K or the other components indicated in Table II which significantly affects sugar content of the juice in a

positive manner. Just what these constituents are is unknown at present, but the problem is being further investigated.

Total Soluble Solids.

T.S.S. × Moisture in pulp. The significant negative correlation (No. 4) shown between these two factors in Table II is presumably due merely to the dilution effect of increased moisture content on concentration of total soluble solids. The same remark would apply to coefficient No. 15, where dry weight of juice is negatively correlated with moisture content of pulp.

T.S.S. × N, K, Ca and Ash. Total soluble solids content is positively correlated with nitrogen content (No. 60), potassium (No. 61) and ash (No. 64), and negatively correlated with calcium content (No. 62). In order to disentangle these correlations and determine which of the factors have an actual effect and which merely an indirect one on T.S.S. content, partial regression coefficients were calculated as before. According to Fisher's " t " table the " t " values of these regression coefficients must exceed 1·987 to be significant at 5% and 2·634 at 1%.

	Partial Regression Coefficients.	" t ' Values.
T.S.S. and N	0·001,946 ± 0·000,481	4·050**
,, ,, K	0·000,754 ± 0·000,563	1·338
,, ,, Ca	−0·008,470 ± 0·004,481	1·890
,, ,, Ash	6·174,765 ± 2·335,530	2·644**

These partial regression coefficients show much the same results as those previously obtained for sugar content, because the largest part of the total soluble solids is made up of sugar. In other words, potassium content does not significantly affect total soluble solids content, whereas high nitrogen content is directly associated with high concentration of soluble solids. As with sugar, there appears to be some undetermined factor in the ash which, when present in increased quantities, causes an increase in total soluble solids.

Moisture Content of Pulp.

Moisture content × N, K, Ca, Ash and Dry weight. Moisture content of pulp is negatively correlated with nitrogen (No. 10), potassium (No. 11), ash (No. 13), dry weight (No. 15) and positively with calcium (No. 12). The partial regression coefficients of moisture content of pulp and the above factors are shown in the following Table, where the " t " values of the partial regression coefficients should exceed 1·987 to be significant at 5% and 2·634 at 1%.

	Partial Regression Coefficients.	" t " Values.
Moisture content and N	−0·031,234± 0·053,673	0·582
,, ,, ,, K	−0·022,318± 0·057,646	0·387
,, ,, ,, Ca	0·517,220± 0·456,87	1·132
,, ,, ,, Ash	−111·584,428±255·708	0·436
,, ,, ,, Dry weight	−36·107,505± 9·971,6	3·621**

The above " t " values show that none of the factors is associated directly with moisture content of pulp except dry weight of juice. It is assumed, however, that this negative relationship between moisture content of pulp and dry weight of juice is merely due to the dilution effect of increased moisture content on the total soluble solids which comprise the dry weight.

NITROGEN CONTENT.

N x Total P, K and Ca. Of the significant correlations which deserve consideration and which may directly affect the nitrogen content of the juice, there are total phosphorus (No. 79), potassium (No. 106) and calcium (No. 107).

The following Table shows the partial regression coefficients and " t " values for the above factors, when the " t " values must exceed 1·987 to be significant at 5% and 2·634 at 1%.

	Partial Regression Coefficients.	" t " Values.
Nitrogen and total P	−0·841,310±0·199,374	4·220**
,, ,, ,, K	0·379,302±0·087,838	4·318**
,, ,, ,, Ca	−2·429,708±0·873,150	2·783**

From the above it appears that all three of the factors, P, K and Ca, have a direct influence on the amount of nitrogen present in the juice.

The more phosphorus or the more calcium present, the less nitrogen is absorbed into the fruit, and vice versa. In the earlier paragraphs dealing with differential treatments in the field, the effect of phosphorus and calcium applications on the absorption of nitrogen was not evident, presumably because insufficient phosphorus and calcium were available for absorption in relation to the larger quantities of nitrogen. The effect of nitrogen on absorption of phosphorus was, however, very marked, because the nitrogen was present in relatively larger quantities than the phosphorus. As indicated in earlier sections also, it might be assumed that if the amount of available phosphorus was sufficiently increased, relatively to nitrogen, then the phosphorus would in turn cause less nitrogen to be absorbed.

The potassium content is associated with nitrogen content in a positive manner, so that increased quantities of K absorbed are directly associated with increased N absorption.

Phosphorus Content.

Total P × N and Ca. Total phosphorus is negatively correlated with nitrogen (No. 79) and positively with calcium (No. 81). The calculation of partial regression coefficients of total P and N and Ca gave the following results, when the "t" values had to exceed 1·987 to be significant at 5% and 2·634 at 1%.

	Partial Regression Coefficients.	"t" Values.
Total P and N	−0·137,727 ± 0·046,858	2·939**
,, ,, ,, Ca	0·605,938 ± 0·453,73	1·325

The above confirms the relationship between N and P referred to several times in earlier paragraphs; the higher the N content the lower the P content, and vice versa.

The positive correlation between Ca and total P (No. 81) must be assumed to be an indirect one, to be explained by the negative correlation between Ca and N (No. 107) and the negative correlation between N and total P (No. 79).

Inorganic P and organic P. By glancing through the coefficients for total P and also for inorganic P it will be noticed that they are always of the same sign and their significance trends are the same. This is to be expected when it is realized that most of the total P is in the inorganic form and the organic P is present in relatively small quantities. This is further borne out by coefficient No. 76 which shows a positive correlation, significant at 1%. between total P and inorganic P.

The negative correlation between organic P and inorganic P (No. 85) might be expected, as these two are the components of one whole, viz. total P.

Coefficients 87 and 94 are of particular interest as they bring out the relationship between nitrogen content and the two forms of P. No. 87 shows a negative correlation between N and inorganic P whereas No. 94 shows a positive correlation between N and organic P. Thus, it appears that a high nitrogen content is necessary for the synthesis of phosphorus-containing compounds, presumably proteins, and when nitrogen content is low more of the phosphorus remains in the inorganic form. This important relationship between N content and the synthesis of P-containing organic compounds is even more clearly illustrated in Table III, where it is shown that wherever the N content

Table III.

Phosphorus Content of Juice expressed as p.p.m. PO_4.

TREATMENTS.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	r
Total Phosphorus	442	341	323	333	329	426	327	314	285	432	352	389	458	310	347	331	363	518	397	365	−0·925**
Inorganic Phosphorus	362	241	221	228	234	319	226	213	188	355	259	288	359	206	237	236	251	434	296	270	−0·938**
Organic Phosphorus	80	100	102	105	95	107	101	101	97	77	93	101	99	104	110	95	112	84	101	95	0·607**
Total Nitrogen	605	1,089	1,118	1,104	1,057	663	1,067	1,072	1,039	654	1,006	833	669	1,082	1,082	1,011	1,061	590	866	986	

r = treatment correlations between Nitrogen and Phosphorus. (These should not be confused with the r's in Table II, which are correlations irrespective of treatment.)

is high the organic P content is also high, even though the total amount of P is simultaneously low.

POTASSIUM CONTENT.

K × total P, N, Ca and Ash. Partial regression coefficients were calculated of K and total P, N, Ca and ash. For significance the "t" values have to exceed 1·987 at the 5% point and 2·634 at the 1% point.

	Partial Regression Coefficients.	"t" Values.
K and total P	0·433,829 ± 0·195,710	2·217*
,, ,, ,, N	0·310,512 ± 0·089,119	3·484**
,, ,, ,, Ca	− 0·927,972 ± 0·826,250	1·123
,, ,, ,, Ash	2,243·656 ±358·796	6·253

The above partial regression coefficients show that nitrogen and ash content directly influence K content ; the higher the N or ash the higher is the K content. This relationship between K and N has also been shown in the partial regression coefficients calculated for nitrogen content in an earlier paragraph. According to the significant "t" value for K and ash above, there appears to be some component in the ash, other than those determined, which also has a direct influence on K absorption.

Considering the frequent references in the literature (18, 17) to the reciprocal effect of Ca and K on the absorption of each other, it seems strange that the above Table of partial regression coefficients does not show a direct influence of Ca on absorption of K. The explanation of this may lie in the fact that many investigators have had insufficient data concerning ions other than the cations with which they were dealing to make it possible to determine by partial regression coefficients which ion really had a direct and which had merely an indirect influence on another ion.

In the present case, for instance, the K content is positively correlated with nitrogen (No. 106), ash (No. 113) and dry weight (No. 114) and negatively with Ca (No. 111). The partial regression coefficients show, however, that the Ca has no direct effect on K. This will be explained in the next paragraph, dealing with Ca content, where the partial regression coefficients will prove that it is the N content which influences the Ca absorption in a negative fashion. It is, accordingly, because N influences Ca and also K that Ca is correlated with K, and this correlation must therefore be looked upon as being an indirect one.

The "t" value of the partial regression coefficient of K and total P shows a relationship between these two factors which is significant at 5%. This is a most unexpected significance when the other correlations discussed here

are considered, and when coefficient No. 80, in Table II, shows quite an insignificant correlation between these two factors. Until more information is available on this point, the above "t" value, significant at 5%, must be accepted with considerable reserve, as it may be due to chance. This insignificant correlation (No. 80) was included in this above calculation of partial regression coefficients merely to bring about uniformity with similar calculations for calcium content given below.

CALCIUM CONTENT.

Ca × total P, N, K and Ash. Calcium content is positively correlated with total phosphorus (No. 81) and negatively with nitrogen (No. 107), potassium (No. 111) and ash (No. 116 ; significant at 5% only, but included here in order to bring about uniformity with the calculation of partial regression coefficients of K given above). The partial regression coefficients and their "t" values of the above factors were calculated and are shown in the following Table. For significance at 5% the "t" values must exceed 1·987 and at 1%, 2·634.

	Partial Regression Coefficients.	*"t" Values.*
Calcium and total P	0·052,473 ± 0·024,711	2·123*
,, ,, ,, N	−0·031,599 ± 0·011,488	2·751**
,, ,, ,, K	−0·014,734 ± 0·013,118	1·123
,, ,, ,, Ash	−53·897,998 ± 53·764,5	1·002

The effect of nitrogen on calcium is most marked. The higher the N content the lower the Ca content, and vice versa. In the above Table and in the preceding one dealing with K, as well as in the Table of correlations (Table II), it will be noted that the N content is associated with the K content in a positive manner and with Ca content in a negative manner. This would prove the negative correlation between Ca and K (No. 111) to be an indirect one, due to the association of Ca and K with N.

As with K, in the preceding paragraph, so now with Ca, the partial regression coefficient of Ca and total P is also significant at 5%. Unfortunately, in this instance too, it is not now possible to assess the value of this significance or to interpret it.

OTHER CORRELATIONS.

Such other correlations as appear in Table II and have not been discussed above are either insignificant or, where there is significance, the correlation is obvious and predictable.

DISCUSSION.

During most of the detailed statistical analysis reported on in this paper only the results of the final year of the investigation have been used. The reason for this is that during the final year all analyses were conducted separately on the material from each replicate of each treatment, so that the statistical interpretation would be as accurate as possible. During the first four years, analyses for the various elements were made only on composite samples representing each treatment and not each replicate of each treatment ; for them, correlations were also calculated and they agreed in all essentials with those presented in Table II, but it was felt that for final conclusions the much more laborious but complete analyses as indicated should be employed.

This paper presents rather vividly the great value of modern statistical methods in the interpretation of biological results. Although many of the conclusions confirm what has been found previously by other investigators, it is felt that even such conclusions are now placed on a more firm and final basis than has been possible by other research workers who either did not have sufficient data or did not acquire them in a manner essential for statistical analysis by the methods employed here.

Various investigators (35, 18, 1, 12, 14, 15, 24, 32, 33, 36, 39) have reported the positive correlation between K and acid and the negative correlation between P and acid. It has been shown in the foregoing correlation coefficients (Table II) that there is also a consistent positive correlation between N and acid content ; it was often due to good fortune or lack of sufficient data that some investigators drew the correct conclusions, because only by the use of partial regression coefficients, as employed here, could the correlations be resolved into those which are directly and those which are indirectly associated, owing to some third common correlation. For instance, it has been reported (1) that applications of ammonium sulphate to orange trees caused an increased acid content in the fruit, except where large quantities of kraal manure were also added. In this instance the acid content can now be explained by the low phosphorus absorption in the first case, where N has been added, and the addition of phosphorus in the manure or the greater availability of phosphorus caused by the manure, in the second case. Other workers (5, 22, 23, 24) also have reported that applications of nitrogenous fertilizers induce an increased acid content in the crop, but this is to be interpreted as merely an indirect effect of the nitrogen, and the direct effect is due to a reduced absorption of P.

The partial regression coefficients of wastage and the many factors correlated with it, form another good illustration of how essential it is to have as complete data as possible before conclusions are drawn, and how necessary it is to distinguish between indirect and direct effects by means of partial regression

coefficients.   It has been claimed, for instance, that high acid content of fruit is associated with low wastage (2), and the implication is that this is cause and effect ; also that low K and low P cause poor storage quality (3, 6) ; the same correlations are shown in Table II, but the partial regression coefficients show that both these factors affect wastage only indirectly. With apples (20), a high sucrose and low nitrogen content was found to favour keeping quality ; a similar correlation is shown for sugar and total soluble solids in Table II, but the partial regression coefficients show these correlations also to be indirect as far as they affect wastage of navel oranges ; on the other hand, with navel oranges, a low N content was always correlated with a high percentage of wastage.   Wallace (38) claims that from the data available, no general statement can be made concerning the relationship of any chemical constituent with storage qualities ; the importance of Ca in metabolism has, however, been emphasized by many investigators (27, 28, 34), and the influence of Ca content on wastage of oranges, as illustrated by the partial regression coefficients given above, is very striking.
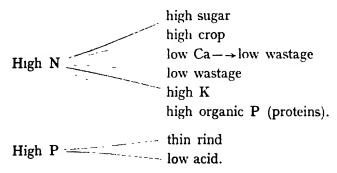
In Table II a significant positive correlation is shown between K and sugar content.   A similar relationship is reported for Satsuma oranges in Japan (32), but the data concerning the latter have not been subjected to intensive statistical analysis, whereas the partial regression coefficients reported in this paper show that the K × sugar correlation is an indirect one only.   Contrary to the result indicated in coefficient No. 100 in Table II, a high N content in grapefruit in Trinidad (18) is claimed to be correlated with a low sugar content ; since the results with grapefruit were not capable of statistical interpretation, it is doubtful whether this apparent contradiction is a real one.

Although none of the factors determined in this investigation shows a direct effect on the moisture content of the pulp, other workers have reported an increase in juice content due to phosphate application (1, 35), and a decrease in juice due to nitrogen applications (1).   It must be remembered, however, that these workers have determined juice content as a percentage of the whole fruit, including the rind, whereas in the work reported here, the actual moisture content of the pulp was determined, excluding the rind.   The explanation for the effect of P and of N on the juice content above referred to is, then, to be sought in the effect of P and of N on the thickness of rind and not on the actual juice content of the pulp itself.   From the correlations and partial regression coefficients reported in earlier paragraphs it is seen that a high N content causes a low P content which, in turn, causes a thick rind.   If, then, the weight of rind is included in a calculation of juice content, as was done by the above workers, it is obvious that a high N application will appear to decrease, and a high P application to increase, the juice of the orange as a whole, whereas the juice content of the pulp, as such, would most probably not have altered at all.

Certain interactions of K, P, N and Ca have been mentioned by many investigators during the last five or six years. Some of these interactions are so very marked that it does not require detailed statistical analysis to prove them, but in the majority of cases it is felt that the data presented on this subject in this paper have finally substantiated what could not have been much more than hypothesis heretofore. Furthermore, most of the conclusions concerning interactions have been drawn by other workers from simple correlations, whereas some such simple correlations are shown in this paper to be indirect, due to some third correlation common to the two first factors. The reciprocal relationship between N and P has been reported on by various investigators (18, 26, 9, 15, 16, 25, 29, 30), but the direct relationship between N and K, as presented here, is seldom satisfactorily reported in the literature (37, 4, 18), and so is the reciprocal relationship between N and Ca (18, 4, 26).

CONCLUSION.

The above report emphasizes throughout the importance of a balanced nutrient solution. In this connection the general conclusion is that, although fundamentally one ion is as important as another, the two most important ions that have to be present in correct proportions and in considerable quantities are $-NO_3$ and $=PO_4$. The proportion and concentration should be such that considerable quantities of both ions should be absorbed, and this would have the following effects :—

High N
- high sugar
- high crop
- low Ca —→ low wastage
- low wastage
- high K
- high organic P (proteins).

High P
- thin rind
- low acid.

SUMMARY.

The importance of plot design and statistical analysis of data is illustrated throughout this paper. The most important conclusions are the following :—

1. Applications of $(NH_4)_2SO_4$ to soil induced very marked increase in weight of crop and number of fruits. There was no significant difference in crop, however, between applications of 2, 4 and 6 lb. $(NH_4)_2SO_4$ per tree.

2. A leguminous cover crop did not increase the N content of the soil, or, accordingly, the size of crop.

3.   Applications of superphosphate, potassium sulphate and lime did not affect size of crop.

4.   The amount of out-season fruit was not affected by any of the treatments.   The size of an in-season crop, however, shows a significant negative correlation with the following out-season crop.   This can be explained on the basis of amount of nutritional reserves.

5.   Although applications of nitrogenous fertilizers caused an increased crop, the increase in tree size did not exceed that of the control, rather the reverse.

6.   Fruits from all plots of all treatments were analysed for P, K, Ca, N, Ash, Dry Weight, Sugar, Total Soluble Solids, Acid, Thickness of Rind and Wastage due to mould.

7.   Correlations and partial regression coefficients were calculated for all factors before final conclusions were drawn.

8.   A high P content and probably also a high Ca content is associated with a low acid content.   On the other hand, a high K content causes a high acid content.

9.   A high K content causes a thick rind of oranges, whereas a high P content induces a thin one.

10.   A high Ca content causes an increase in the amount of wastage due to mould during storage.   Nitrogen has the reverse effect.

11.   None of the factors determined has any effect on sugar content except N, which increases sugar content with increased N content, and ash, which has a similar effect.

12.   Remarks similar to those for sugar apply to total soluble solids.

13.   The juice content of the pulp is not affected directly by any of the factors determined.

14.   There is a reciprocal relationship between P and N, and between Ca and N ; there is also a direct positive relationship between K and N, and between K and ash.

15.   High N is positively correlated with high organic P, irrespective of the amount of total P present.   High N is therefore necessary for synthesis of phosphatic organic substances.

ACKNOWLEDGMENTS.

In connection with the vast amount of chemical analyses and the recording of other data during the six year period, the valuable services of E. L. Marais, A. J. v.d. Merwe, A. C. Bathurst, E. M. Nyenhuis, O. T. van Niekerk, T. C. de Waal and N. H. Shaw are acknowledged.

The African Realty Trust, on whose estate these fertilizer trials were carried out, are thanked for their generous co-operation in connection with the field work throughout this investigation.

The South African Co-operative Citrus Exchange financed the purchase of fertilizers and fruit throughout the period, and it is a pleasure to acknowledge this assistance here.

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BOOK REVIEWS

AN OUTLINE OF CYTOLOGICAL TECHNIQUE FOR PLANT BREEDERS.
Imperial Bureau of Plant Genetics. (Cambridge. 1s. 6d.)

As Sir Daniel Hall says, in a brief Foreword to this Bulletin, a knowledge of cytology and some acquaintance with its methods has become essential to the plant breeder. The Bulletin aims at giving an account of the standard methods used in plant cytology based on practical experience. After an introduction dealing with the value of cytology in plant breeding and some general remarks on methods, the Bulletin describes the paraffin method (including staining with iron alum-haematoxylin and with gentian violet), the aceto-carmine method and smears with standard fixatives and stains. Hints on the use of the microscope, a list of fixatives, with formulae, and a short list of literature references are given. The Bulletin is suitable not only for plant breeders but also for anyone wishing to learn standard methods.

THE PESTS OF FRUITS AND HOPS. By A. M. Massee, D.Sc. (Crosby, Lockwood & Son Ltd., London, 1937. pp. 294; 26 plates. Price 15/-.)

For nearly thirty years, Theobald's " Insect Pests of Fruit " has been invaluable as *the* book of reference on the subject, but there can be no question that a new account of the insect and allied pests of fruit and hops and of present-day methods for their control was badly needed. No one is better qualified than Dr. Massee to undertake the task of filling this evident gap and the appearance of his book is very welcome.

It is essentially a practical book. The chief fruit crops are considered in turn, each chapter beginning with a list of the pests, giving the parts of the plant attacked and a note of the page on which the pest is discussed, so that reference to any particular insect can quickly be made. A short general account of each pest is followed by notes on the life cycle and sections on recognition in the field and on control measures. Detailed descriptions of insects are, however, omitted and the author has succeeded in avoiding almost completely the use of technical terms, without loss of accuracy. The information necessary for identification of the pests in the field is very clearly presented, emphasis being laid rather on the nature of the injury caused than on the appearance of the pest concerned, and the text is amplified by the numerous admirable photographs contributed to the book by Mr. R. M. Greenslade. Many insects which are not normally important enough from an economic point of view to justify employment of control measures are dealt with briefly and it is valuable to have

these included, for " minor " pests may and do sometimes become " major " pests if only locally. Finally, there are chapters on Beneficial and Harmless Insects, on Insecticides and on Spraying Equipment and Methods, the last written by Mr. J. Turnbull; and seperate general indexes of the popular and scientific names of the pests are provided. The book is well produced and there is a noticeable absence of misprints.

Readers of Dr. Massee's book cannot fail to appreciate the notable advances that have taken place in recent years in the measures available for control of many of our most important fruit pests and, as pointed out in the preface, the co-operation of chemists with biologists is an important factor contributing to this advance. It is satisfactory that he is able to write (p. 244):—

> " Thanks to the modern methods of pest control, the Hop-Damson Aphis, or ' Hop Fly ', is no longer a continual source of worry to the hop grower during the summer months. Until some ten years ago, however, it was undoubtedly one of the most important insect pests associated with hops, while the damage it caused to damson and plums was no less severe. . . .
>
> " To-day the Hop-Damson Aphis is a very secondary pest, and it is very rarely met with in a well-kept garden " ;

and there are similar remarks with respect to Apple Aphides, Apple Sucker, Black Currant Gall Mite and other well-known pests. On the other hand, there remain an abundance of unsolved problems, and the unsatisfactory nature of present control measures for certain common pests—as, for example, the Apple Blossom Weevil—and the insufficiency of our information about others, such as Strawberry Eelworm, are frankly admitted. In the chapter on beneficial species, attention is specially drawn to predacious insects which are of more importance than is commonly realized and to the unfortunate effect of certain control measures in reducing their numbers, a subject that might well receive more adequate investigation.

It would be easy, but unnecessary in this Journal, to continue at length enumerating points of particular interest. Reference must, however, be made to the short account of insecticides and their application in Chapter XIV. Growers especially will find this of interest and value. The chemical aspect of the subject is not touched on, but the various types of oil washes, lime sulphur, lead arsenate, nicotine, derris and pyrethrum, as well as soft soap and other wetters and spreaders, are briefly discussed in an exceedingly practical manner. The author is uncompromising in his opinion that tar distillate washes should be applied every year. He says (p. 263):—

"In view of the number of spray applications that are recommended to-day, the commercial growers frequently inquire whether it would be a practical proposition to omit the tar oil in some seasons. It would not " ;

and proceeds to give his reasons. The need for caution in using summer petroleum washes owing to the risk of damage to foliage and fruit is emphasized, but the author anticipates that the present tar-petroleum mixed washes will be improved before long and the number of pests controllable by one spraying in the winter thus increased. Some day, insecticides may become unnecessary and we may find out how to grow plants highly resistant to all pests and diseases. "Pending this millennium, however, we have to make the best of whatever knowledge is available, and in its application the sprayer still of necessity plays a principal part."

Dr. Massee writes clearly and concisely and almost every page bears evidence of his close personal acquaintance, both in the field and under laboratory conditions, with the habits of the pests he is describing. This gives to the book a special individual character and it is very far removed from a mere compilation of facts, though the literature of the subject and the work of other investigators has by no means been neglected. The author's special knowledge of the problems of pest control as they affect fruit-growers in Kent has naturally influenced his treatment of the subject, but he has put entomologists and growers everywhere in his debt by placing at their disposal this authoritative account of the pests of fruit and of hops. It is a pleasure to commend the book to the attention of all interested in fruit growing.

C.T.G.

SCIENTIFIC HORTICULTURE. Edited by R. T. Pearl. Vol. 5, pp. 196. Wye: South-Eastern Agricultural College, February, 1937. Price 3s. 6d. net (postage 5d. extra).

In this Volume research workers will be interested in the two articles by T. Whitehead and the late W. M. Davies, of University College, Bangor, on virus problems in relation to seed potato production in North Wales. R. W. Marsh, Long Ashton, contributes a refreshingly concise account of recent American work on copper fungicides. F. Kidd and C. West, Cambridge, describe the main phases in the development of the Bramley apple, and present experimental data in support of the tradition that the right time to pick the apple for storage is when it will yield to a gentle twist of the stalk. Prof. R. H. Stoughton, Reading, in reviewing the problem of bud dormancy, surveys the field of research without encroaching unduly on the ground covered elsewhere in this Volume by O. N. Purvis, London, in an article on recent Dutch investi-

gations into the temperature requirements of hyacinths, and by M. Thomas, Newcastle, in a discussion of recent work on plant hormones.

W. J. C. Lawrence contributes an interesting summary of twenty-five years' research at the John Innes Horticultural Institution, to which an appendix by Miss B. Schafer is added containing a report-summary of current work there. On the more strictly practical side, W. Corbett outlines the history of the pot plant industry in this country, whilst other descriptive articles deal with commercial horticulture in Essex and Middlesex by H. Fraser and P. E. Cross, respectively, and growing fruit in North Wales by H. Taylor. B. S. Furneaux and W. G. Kent have an interesting note on the cause of a collapse of fruit trees shortly after coming into leaf, locally known as " The Death " and due to root suffocation.

The Volume covers a wide range and provides up-to-date information on many horticultural subjects of particular interest to research workers, advisory officers and students.

N.B.B.

HORTICULTURAL ASPECTS OF WOOLLY APHIS CONTROL TOGETHER WITH A SURVEY OF THE LITERATURE. By R. M. GREENSLADE. (Tech. Comm. Imperial Bureau of Fruit Production, East Malling, Kent, England, 8, 1936, pp. 88, bibls. 555 (general) and 156 (biologic control). 2s. 6d.)

The voluminous literature published during the last hundred years on Woolly Aphis or American Blight and the divergent opinions expressed in it have long demanded examination and critical analysis. In November, 1933, a Memorandum and Questionnaire on the incidence and control of the pest was circulated by the Imperial Bureau of Fruit Production to all the apple growing countries of the world and co-operation invited.

The literature and the replies to the Questionnaire have been thoroughly sifted by the author, who, having devoted himself for some years to the Woolly Aphis problem in its many aspects, is in an exceptionally good position for the task.

First, the insect and its habits, its spread in the orchard, its methods of feeding and its possible alternative hosts are dealt with. Next, the bearing of climatic factors, temperature, humidity, wind and sunlight, on its incidence is considered. Control measures are considered in detail: (1) Artificial control including spraying, fumigation, tree injection, cultural practice, etc. ; (2) Natural control by *Aphelinus mali* and other parasites ; (3) Control by use of resistant stocks and varieties. A particularly interesting account is given of existing resistant varieties, of the breeding work in progress in England and

of the few indications afforded as yet of the possible causes of resistance ; (4) Control by legislation, e.g. quarantine measures, etc.

The literature from nearly 300 sources is further dealt with in two annotated lists which follow.   The first, general, contains 555 references, while the second contains 156 references to articles on the biological control of the pest.

Finally, the Memorandum and Questionnaire mentioned above are reproduced in full, and a list of persons who replied is given. This list forms, incidentally, a useful index of workers interested in the subject.

Both to investigators and growers this publication should prove of great value.

VEGETATIVE PROPAGATION OF TROPICAL AND SUB-TROPICAL FRUITS. By G. St. Cl. Feilden and R. J. Garner. (Tech. Comm. Imperial Bureau of Fruit Production, East Malling, Kent, England, 7, 1936, pp. 67, bibl. 123.   2s.)

This compilation forms the second of a series of articles issued by the Imperial Bureau of Fruit Production on the vegetative propagation of tropical and sub-tropical horticultural crops, the first on citrus having appeared in 1932.

It is based on a thorough examination of existing literature and on the answers to enquiries sent to many workers in the tropics.   It is in two parts. In the first will be found an illustrated and simple account of the different operations of budding, grafting, etc., referred to later.   This is very welcome, in view of the looseness with which certain propagation terms are used in different parts of the English speaking world.   An account follows of the methods commonly recommended for use or trial in the asexual propagation of some one hundred types of fruit, and references are given to the source of the information in each case.

The bibliography concerned precedes a useful index in which both common and scientific names of the plants discussed are included.   The publication should prove a useful reference work to the tropical horticulturist.

STUDIES ON NEW VARIETIES OF APPLE ROOTSTOCKS

By H. M. TYDEMAN

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IT is now almost twenty years since a joint programme of breeding and testing new varieties of apple rootstocks was arranged between the East Malling Research Station and the John Innes Horticultural Institution. Hatton (1, 2, 3, 4) had already made considerable progress in his studies of the influence of the sixteen varieties of so-called " Paradise " rootstocks, collected at East Malling, upon apple scions, and was in a position to forecast something of the limitations as well as the potentialities of this series. The early interest in the genetics of fruit trees of Prof. Bateson and his assistant, Mr. M. B. Crane, made possible the joint arrangement whereby the facilities for plant breeding work, available at the John Innes Horticultural Institution, could be used for making crosses between the various rootstocks, the seedlings from which were sent to East Malling later to be tested as possible rootstocks. Thus, while the specialist knowledge of plant breeding methods possessed by Mr. Crane could be utilized in raising the families of seedlings, their testing could be undertaken at East Malling, where experience in comparing the different races of rootstocks had been accumulating.

Almost from the beginning, work was directed towards two somewhat dissimilar objects. On the one hand, a large number of crosses were made between certain Malling selected " Paradise " rootstocks and varieties like Northern Spy, which were reputed to be immune from attack by Woolly Aphis. It was hoped that new races of rootstocks would be originated, combining the wide range of influence over the scion and ease of multiplication possessed by certain members of the " Paradise " series, with immunity from attack by Woolly Aphis. A considerable literature dealing with this work now exists (5, 6, 7, 8).

From 1917 onwards, crosses were made between various Malling selections of " Paradise " rootstocks. From the economic point of view, crosses were directed towards the combination in single seedlings of complexes of characters borne separately in the existing varieties of rootstocks, or the origination of new characters of value. At the same time it was hoped that such crosses would yield information of genetic interest.

The present paper deals with seedlings from a cross, made in 1923, by Crane, between the French Paradise (Malling No. VIII) and Jaune de Metz (Malling No. IX). The two rootstocks stand quite apart from the rest of the

Malling series of " Paradise " because of their relatively extreme dwarfing effect on the scion.   This is strikingly associated with " precocity ", that is, the capacity to bear prolific quantities of blossom and comparatively heavy crops of fruit relatively early in the life of the tree.   Although they exert an almost identical influence on the scion, Malling No. IX has almost completely superseded Malling No. VIII in commerce as the typical " dwarfing " root-stock, because the latter is very susceptible to apple Scab and Mildew.   Crane's principal object in making the cross was to study the inheritance of the dwarfing character in the unworked seedlings.   They were sent to East Malling in 1926, because it was thought that they might include valuable new dwarfing and semi-dwarfing rootstocks.

The Trials.

Altogether 136 seedlings from this cross were tested to ascertain the ease with which they could be vegetatively propagated.   Ultimately, nineteen were selected as showing good promise.   These were then multiplied in sufficient quantities for budding in order to obtain information as to their influence upon the scion.   The scion used throughout was Lane's Prince Albert, because it was already known to be a quick indicator of differential rootstock effects. Three trials were laid out as follows : —

Trial A.   Comprising ten rootstocks, budded in 1930.   There was a unit of between fifteen and thirty-five trees on each rootstock and forty-five trees on Malling No. IX.

Trial B.   Containing fifty trees on each of nine rootstocks, budded in 1932, with control trees on Malling Nos. IX and VII.   Trees on No. VII were included because it was hoped to find among the seedlings, rootstocks com-bining the early vigour and precocity characteristic of this variety, but without its faults.

Trial C.   A unit of forty trees on each of the nineteen rootstocks was budded in 1933 for a complete trial.   Trees on Malling Nos. IX and VII were again included.   The results of this trial will be discussed most fully, but those from the preliminary Trials A and B have been included because they are to a large extent confirmatory.

The Method of the Intensive Trial.

Since it was desired to test a considerable number of these new rootstocks, it was decided to devise a special method for testing them on an intensive scale.   They were planted out in nursery rows at 5 to 6 feet apart, the rootstocks being from 2 to 2½ feet apart in the rows, and were budded in situ. Latterly, it has been usual to insert more than one bud into each rootstock in

order to eliminate bud failures, with the consequent disturbing effect on the experiment of gaps in the rows. The trees were allowed to grow where they were budded, being left unpruned or only lightly regulated. They were trained to canes and wires in their early years and, where they showed signs of becoming crowded at the end of a few years, alternate trees were removed without disturbing the roots of adjacent trees. This process of removing alternate trees was repeated at intervals until, at the end of six years, the trees in the oldest of these trials were spaced at intervals of eight or ten feet.

For this intensive type of trial it was necessary to have a comparatively large unit of trees. Thus, in the 1932 trial, there was a unit of fifty trees on each rootstock and, in the complete trial budded in 1933, units of forty trees. In both trials the trees were planted in blocks of ten and randomized in five, and in four, different positions on the plantation respectively.

THE RECORDS.

The observations and records made on these trees were designed to provide information as to the influence of the rootstock on (1) the vigour, (2) the productivity, and (3) the health of the scion.

I. THE INFLUENCE OF THE ROOTSTOCK ON THE VIGOUR OF THE SCION.
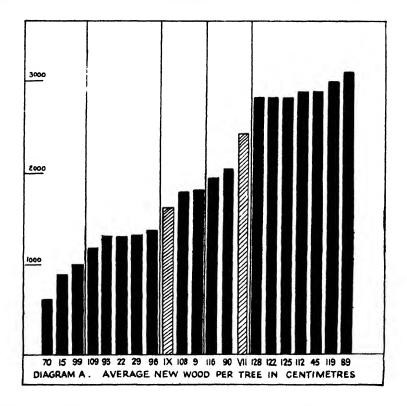
(a) As MEASURED BY THE LENGTH OF NEW WOOD.

The length of new wood was measured each winter, and the records are summarized in Table I. The most notable feature is the extremely wide range of variation in the vigour which these rootstocks have imparted to the scion. Thus, in Trial C, during three seasons, while the trees on No. 70 had made only 718 cm. of new wood, those on No. 89 had made 3,127 cm. The trees on the remaining seventeen rootstocks ranged themselves between these two extremes. The variation, however, does not appear to be strictly continuous. This is shown well in Diagram A.

Thus, trees on the first three rootstocks are separated by a considerable interval from the next five which, in turn, are considerably less vigorous than the next three, which include the sets of trees on Malling No. IX. Trees on Nos. 116 and 90 are, in turn, considerably less vigorous than the trees on the remaining seven rootstocks. It was possible, on the basis of these differences, to separate the rootstocks into five groups. This grouping is confirmed by a statistical analysis of the totals of new wood for 1936 of the trees in Trial C, and although there are some exceptions, is supported by the records of the trees in Trials A

and B.   At the same time it should be pointed out that this result may possibly
have been due to the comparatively small number of rootstocks studied and
that had the numbers been larger, the variation might have been found to have
been continuous.   The extreme differences in vigour are well illustrated in
Figs. 1 and 2.

Since units of trees on Malling No. IX were planted for comparison in all
three of these trials, and trees on Malling No. VII were planted in the two
more recent trials, B and C, it is possible to make a direct comparison between
the trees on the VIII × IX rootstocks and those on the two varieties of

70 15 99 109 93 22 29 96 IX 108 9 116 90 VII 128 122 125 112 45 119 89
DIAGRAM A .   AVERAGE NEW WOOD PER TREE IN CENTIMETRES

" Paradise ".   Table I shows that trees on eight of these rootstocks, in the
complete Trial C, had made less wood during their first three years than had
those on Malling No. IX, the least vigorous of the Malling series, while eleven
of them had made a significantly greater amount of new wood.   The two older
trials do not contain the complete range of rootstocks, and it is less easy to
assign a definite position to the trees on Malling No. IX.   In Trial A, the trees
on three of the rootstocks had made a smaller aggregate of new wood, on the
average, by the end of their sixth year, than had those on Malling No. IX ;
and those on seven rootstocks had made a greater amount, although trees on
two of them, Nos. 93 and 22, had been assigned to a less vigorous group on
the basis of the new wood totals of younger trees.   The trees in Trial B have

TABLE I.

Average New Wood per Tree in Centimetres.

Rootstock No.	Trial A. Budded in 1930. Six years total.	Trial B. Budded in 1932. Four years total.	Trial C. Budded in 1933. Three years total.
Group 1.			
70	—	—	718
15	—	—	896
99	—	403	997
Average :	—	**403**	**871**
Group 2			
109	1,873	—	1,155
93	2,670	—	1,330
22	2,954	—	1,332
29	1,771	503	1,350
96	—	—	1,395
Average :	**2,324**	**503**	**1,313**
Group 3			
IX	2,239	458	1,644
108	3,176	530	1,801
9	2,107	359	1,833
Average :	**2,508**	**449**	**1,760**
Group 4			
116			1,957
90	-	785	2,025
VII		541	2,462
Average :	-	**664**	**2,148**
Group 5.			
128	-	774	2,838
122		—	2,844
125	9,818	951	2,855
112	6,902	1,060	2,937
45	5,624	1,134	2,966
119	-	—	3,057
89	8,000	—	3,127
Average :	**7,588**	**980**	**2,946**

made very unsatisfactory growth throughout and, by the end of their fourth year, those on Malling No. IX had actually made little more than a quarter of the new wood made in three years by the trees on Malling No. IX in Trial C. In spite of this, the trees on the rootstocks represented in Trial B had, with few exceptions, ranged themselves in the same relative order as the trees on the same rootstocks in Trial C. As was expected, the trees on Malling No. VII made considerably more new wood than those on Malling No. IX. It is noteworthy, therefore, that, in Trial C, trees on seven of the selections from

VIII × IX had made, on the average, a greater amount of new wood by the end of their third year.

Although it is impossible to make any direct comparison of the relative vigour of the trees on the VIII × IX seedlings and those on the sixteen varieties of " Paradise " included in Hatton's original trials, since neither cultural nor seasonal conditions were similar, there are grounds for supposing that the more vigorous rootstocks in both series were about equally vigorous by the end of their third year. Trees of Lane's on the dwarfing Malling No. IX, in

TABLE II.

Diameter of Stem in Centimetres.   Average per Tree.

Rootstock No.	Trial A. Budded in 1930 At six years old.	Trial B. Budded in 1932. At four years old.	Trial C. Budded in 1933. At three years old.
Group 1.			
70	—	—	1·73
15	—	—	1·97
99	—	1·51	1·74
Average :	—	**1·51**	**1·81**
Group 2.			
109	3·02	—	2·23
93	3·64	—	2·29
22	3·61	—	2·49
29	3·14	1·66	2·16
96	—	—	2·18
Average :	**3·36**	**1·66**	**2·27**
Group 3.			
IX	3·23	1·74	2·47
108	3·94	1·79	2·48
9	3·05	1·63	2·33
Average :	**3·41**	**1·72**	**2·42**
Group 4.			
116	—	—	2·80
90	—	2·05	2·74
VII	—	1·98	3·12
Average :	—	**2·01**	**2·89**
Group 5			
128	—	2·36	3·37
122	—	—	3·33
125	6·58	2·52	3·35
112	5·83	2·45	3·29
45	5·51	2·46	3·51
119	—	—	3·58
89	6·03	—	3·49
Average :	**5·98**	**2·45**	**3·42**

Hatton's trial,* made 49% of the wood made by the trees on the very vigorous Malling No. XII ; trees on No. IX in this trial made 52% of the wood made by the trees on No. 89, the most vigorous of these seedlings. Again, trees on the semi-dwarfing Malling No. VII, in Hatton's trial, made 84% of the wood made by those on No. XII, while, in this trial, trees on No. VII made 79% of the wood made by trees on No. 89. By the end of their sixth year, the similarity is less close. Trees on No. IX in Hatton's trial, made 20% of the wood made by those on No. XII, but, on No. IX, in this trial the trees made 28% of the wood made by those on No. 89. The results of such comparisons should, however, be received with the greatest caution since the very different conditions under which the two trials were carried out may have had a greater effect on certain rootstocks than on others, which would have affected their relative positions within the series.

(b) As Measured by Diameter of Stem.

The records include measurements of the thickness of the stem at a uniform height above the union. They are summarized in Table II.

The rootstocks are arranged in ascending order of vigour as expressed by new wood. In general, stem diameter appears to have been an equally efficient measure of the relative vigour of these trees. Table II shows that while there are minor alterations in the relative positions of the rootstocks within the groups, only Groups 2 and 3 overlap. Thus, the trees on No. 22, in Group 2, Trial C, were slightly stouter, on the average, than those on any of the rootstocks in Group 3, while at six years old, in Trial A, the trees on Nos. 93 and 22 were stouter than those on the two rootstocks Malling No. IX and No. 9, in Group 3. In Trial C the trees on Malling Nos. IX and VII retained their relative positions in Group 3 and 4 respectively. With slight exceptions, there appears to have been a very close correlation between new wood and stem thickness throughout.

(c) As Measured by Weight of Tree.

The weight of the tree is, perhaps, the most complete expression of tree size obtainable. The periodical thinning out of the trees in these trials has made it possible to record the weight of the trunk and branches at various ages. In Table III are summarized the trunk and branch weights of from seven to fifteen trees on each rootstock, removed at five years old from Trial A, and of thirty trees on each rootstock, in Trial B, removed at four years old. In the

* See R. G. Hatton, " The Influence of Different Rootstocks upon the Vigour and Productivity of the Variety Budded or Grafted Thereon." Journ. Pom. & Hort. Sci., 1927, 6, Appendix A.

TABLE III.

Weight of Tree at Removal.

Rootstock No.	Trial A. Removed at five years old in lbs.	Trial B. Removed at four years old in lbs.
Group 1.		
99	—	1·18
Average :	—	**1·18**
Group 2.		
109	2·16	—
93	2·51	—
22	3·26	—
29	2·05	1·25
Average :	**2·50**	**1·25**
Group 3.		
IX	2·56	1·41
108	2·46	1·67
9	3·00	1·04
Average :	**2·68**	**1·37**
Group 4.		
90	—	2·06
VII	—	1·79
Average :	—	**1·93**
Group 5.		
125	5·46	2·38
112	5·80	2·50
45	5·11	2·54
89	5·40	· ·
128	—	2·27
Average :	**5·44**	**2·42**

latter, the weights are of entire trees, including the root systems.   These figures provide an almost perfect confirmation of the data on tree size already given. In Trial A, in spite of slight overlapping between the trees on the rootstocks in Groups 2 and 3, vigour of tree as expressed by weight shows very close correspondence with vigour as expressed by new wood and diameter of stem. In Trial B, the only anomalous case is that of trees on seedling No. 9, in Group 3, which were lighter, on the average, than those on No. 29, in Group 2, or No. 99, in Group 1.   Since, however, the trees on this rootstock had made less new wood by the end of their fourth year than the trees on the other two rootstocks, this result was only to have been expected.

It is a little remarkable that a cross between two such rootstocks as Malling Nos. VIII and IX, which have been shown to induce so pronounced

a dwarfing effect on apple scions, should have given rise to seedlings showing so wide a range of variation in their influence upon the vigour of Lane's Prince Albert, at least during the early years. At one extreme, eight rootstocks have made trees less vigorous than those on Malling No. IX, up to the end of their third year; indeed, trees on the most dwarfing of them, No. 70, had made less than half the wood growth made by those on No. IX. At the other, seven rootstocks were definitely more vigorous than Malling No. VII, a fairly vigorous rootstock during its early years. This result appears to provide another illustration of the extremely heterozygous condition of apple varieties; it also proves that potentialities for vigour must exist in such dwarfing rootstocks as Malling Nos. VIII and IX, probably due to complementary factors borne separately by the two varieties, which can come together only when they are crossed.

TABLE IV.

Illustrating the Relation between the Size of the Swelling at the Union and the Average Size of the Tree.

Rootstock No.	Diameter at Union in cm.	Diameter at 3 in. above Union in cm	Percentage Increase
Group 1			
99	4·10	1·67	145·6
Average :	**4·10**	**1·67**	**145·6**
Group 2			
29	4·37	1·86	136·2
Average :	**4·37**	**1·86**	**136·2**
Group 3.			
IX	4·62	1·93	139·8
108	3·93	1·98	100·2
9	3·55	1·62	143·2
Average :	**4·03**	**1·84**	**127·7**
Group 4.			
90	4·94	2·30	116·6
VII	3·11	1·91	63·2
Average :	**4·03**	**2·11**	**89·9**
Group 5.			
128	3·77	2·34	61·6
125	3·91	2·59	50·5
112	4·72	2·75	71·8
45	4·16	2·72	53·7
Average :	**4·14**	**2·60**	**59·4**

(d) The Relation between Size of Tree and the Swelling at the Union.

A noticeable characteristic of the trees on many of the types of " Paradise " rootstocks is the presence of a large swelling at the point of union. This is particularly pronounced on trees on the dwarfing rootstocks Nos. VIII and IX and on No. IV, a vigorous rootstock in its early years. This swelling increases in size more rapidly than the rest of the tree and, in extreme cases, may prove very unsightly. Such swellings were very common on the trees on certain of the rootstocks of the VIII × IX series. Since they appeared to occur more

Table V.

Average Number of Blossom Trusses per Tree.

Rootstock No.	Trial A. Budded in 1930. Six years total.	Trial B. Budded in 1932. Four years total.	Trial C. Budded in 1933. Three years total.
Group 1.			
70	—	—	76·7
15	—	—	72·0
99	—	81·8	78·3
Average :	—	**81·8**	**75·7**
Group 2.			
109	340·3	—	64·0
93	421·3	—	71·7
22	387·0	—	69·9
29	395·1	75·0	89·3
96	—	—	77·6
Average :	**385·9**	**75·0**	**74·5**
Group 3.			
IX	421·1	81·9	81·3
108	500·8	98·9	78·6
9	433·1	70·6	69·0
Average :	**451·7**	**83·8**	**76·3**
Group 4.			
116	—	—	66·0
90	—	97·7	57·2
VII	—	90·9	40·7
Average :	—	**94·4**	**54·7**
Group 5.			
128	—	85·1	46·2
122	—	—	53·5
125	830·0	88·9	39·2
112	707·3	102·9	47·7
45	606·0	97·5	62·0
119	—	—	49·1
89	566·3	—	51·3
Average :	**680·2**	**93·6**	**49·8**

commonly on trees growing on the less vigorous rootstocks, it was thought probable that the size of the swelling was closely associated with tree size. Measurements of the sizes of the swellings on nine of these rootstocks were made when the trees were four years old. The diameters of the stems at the point of union and at three inches above were measured. The percentage difference between the diameters at these two points was taken as the measure of the relative size of the swelling. The average figures for units of ten trees on each rootstock are shown in Table IV. The rootstocks are arranged in order of increasing vigour as measured by new wood. With only slight exceptions, the size of the swelling decreases with the increasing size of the tree. Although there were minor variations, in general, the size of the swelling was found to be very constant from tree to tree on the same rootstock.

II. THE INFLUENCE OF THE ROOTSTOCK ON THE PRODUCTIVITY OF THE SCION.

(a) As Measured by Blossom Production.

While it will be obvious that the ultimate criterion of the relative productiveness of fruit trees must lie in a comparison of the crops of fruit which they are capable of carrying, the incidence of adverse weather conditions, premature dropping and the depredations of insect pests, may seriously impair the value of such records. It has always been thought advisable, therefore, to supplement the data on actual fruit production, with counts of the blossoms borne.

The number of trusses were counted each year. The records are summarized in Table V. They show that, at least up to the end of their third year, blossom production was closely associated with the dwarfing character in the trees on these rootstocks. Although the trees on No. 29, in Group 2 (Trial C), had actually produced a larger number of trusses than had those on any other rootstock in the series, and the trees on No. 108 in Group 3 had borne a larger number than had the trees on any of the rootstocks in Group 1, a fairly consistent reduction in the average number of trusses occurs between the trees on the least vigorous and those on the more vigorous rootstocks. This tendency becomes very marked among the more vigorous rootstocks in Groups 4 and 5. At the end of six years, however, this result had been completely reversed and the trees on the four vigorous rootstocks represented (Group 5 in Trial A), had produced about twice as many trusses as had those on the four dwarfing rootstocks in Group 2. This result had unquestionably been brought about at least in part by the greatly increased size, and consequently greater blossom bearing surface, of the trees on the vigorous rootstocks.

(b) The Relation between Blossom Formation and Tree Size.

While the foregoing data illustrate very clearly the differences found in the actual number of trusses, since the trees differed widely in size, it is clear that the figures in Table V do not provide an adequate picture of the relative precocity of the trees. Thus, the much greater number of trusses borne by the trees on the vigorous rootstocks, at six years old, when compared with the trees on the more dwarfing rootstocks, may have been entirely due to their much greater size, or may indicate that the trees on the more vigorous

TABLE VI.

Average Number of Blossom Trusses per 100 centimetres of New Wood.

Rootstock No.	Trial A. Budded in 1930. Six years average.	Trial B. Budded in 1932. Four years average.	Trial C. Budded in 1933. Three years average.
Group 1.			
70	—	—	16
15	—	—	12
99	—	26	14
Average :	—	26	14
Group 2.			
109	20	—	12
93	18	—	10
22	15	—	11
29	24	19	12
96	—	—	13
Average :	20	19	12
Group 3.			
IX	20	21	9
108	16	22	9
9	33	26	7
Average :	23	23	8
Group 4.			
116	—	—	7
90	—	14	6
VII	—	18	3
Average :	—	16	5
Group 5.			
128	—	12	3
.122	—	—	3
125	9	12	3
112	10	12	3
45	12	10	3
119	—	—	3
89	8	—	4
Average :	10	12	8

rootstocks were entering on a definitely more productive stage by their sixth year. In order to make possible a comparison of the actual productiveness of the trees on the different rootstocks, irrespective of their size, the average number of trusses borne per hundred centimetres of new wood made have been calculated and the results for the three trials are shown in Table VI. When treated in this way the six year old trees show a decrease in productiveness with the increasing vigour of the rootstocks similar to that shown by the trees at an earlier age. Thus, the trees on No. 29 in Group 2 bore an average of twenty-four blossom trusses per 100 centimetres of new wood, while those on No. 89 in Group 5 bore only eight. The Table illustrates very clearly the much greater productiveness of the trees on the rootstocks in the extra dwarf groups, 1 and 2, when compared with those in the third and subsequent groups.

These figures lend some support to the view that, with advancing age, the trees on the more vigorous rootstocks were becoming increasingly productive as compared with those on the more dwarfing rootstocks. Thus, if minor variations are excluded, the five rootstocks in Group 2 (Trial C) bore, on the average at three years old, just four times as many trusses per centimetre of wood as did the seven rootstocks in Group 5 (Trial C) ; but, at six years old (Trial A), trees on the rootstocks in the former group bore only twice the number of trusses per centimetre of new wood borne by the trees in the latter group.

It does appear, nevertheless, that the trees in the more vigorous Group 5 are relatively much less productive assessed on this basis, even at six years old, than are those on the rootstocks in the more dwarfing Groups 2 and 3, and that the much greater aggregate blossom formation of the trees on the more vigorous rootstocks, by the end of their sixth year, is to be attributed chiefly to the relatively greater areas of potential blossom bearing surface available and, only to a minor degree, to a slight increase in the inherent productiveness, with advancing age, of these trees.

(c) EARLY EVIDENCES OF PRECOCITY.

In the preceding section it has been shown that the correlation between early inherent productiveness and the dwarfing effect, which is, in general, markedly shown in the Malling series of " Paradise " rootstocks, can be extended beyond Hatton's dwarfing group, represented in these trials by Malling No. IX, to the extra dwarfing rootstocks obtained in some of the seedlings in the family raised from VIII × IX. As may be seen from Table V, the trees on the rootstocks in Groups 1, 2 and 3, in Trial C, bore, over their first three years, on the average, an approximately equal number of trusses, and the superior productiveness of the trees on the rootstocks in Group 1 compared with those in Group 2, or of those in Group 2 when compared with the trees in Group 3, cannot be shown, even at this relatively early stage, until the differential effect

of tree size has been eliminated. It is, however, possible to demonstrate the greater actual productiveness of the trees on the rootstocks in these dwarfing groups, in spite of their smaller size, at an earlier stage. In Table VII the average

TABLE VII.
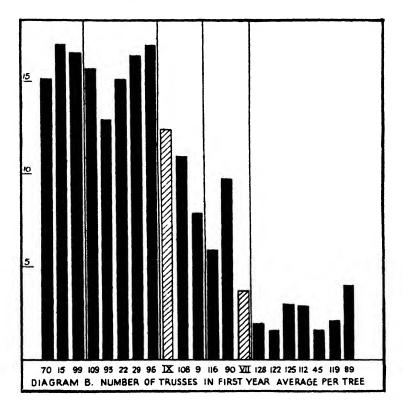
Average Number of Blossom Trusses in First Year.

Rootstock No.	Trial A. Budded in 1930.	Trial B. Budded in 1932.	Trial C. Budded in 1933.
Group 1.			
70	—	—	15·2
15	—	—	16·9
99	—	16·6	16·4
Average :	—	**16·6**	**16·2**
Group 2.			
109	2·2	—	15·7
93	1·0	—	13·1
22	2·7	—	15·3
29	1·8	13·9	16·5
96	·	—	17·2
Average :	**1·9**	**13·9**	**15·6**
Group 3.			
IX	4·0	13·1	12·5
108	0·8	15·6	11·3
9	0·0	11·9	7·9
Average :	**1·8**	**13·5**	**10·6**
Group 4.			
116	—	—	6·2
90	—	9·2	9·8
VII	—	8·9	3·7
Average :	—	**9·1**	**6·6**
Group 5.			
128	—	6·3	2·8
122	—	—	1·6
125	1·5	7·2	3·0
112	1·1	7·8	2·9
45	0·1	5·2	1·6
119	—	—	2·2
89	0·9	—	4·1
Average :	**0·9**	**6·6**	**2·6**

number of trusses per tree, borne in the spring following the end of their maiden year by the trees on all these rootstocks are shown. The result is perhaps more striking in diagrammatic form and, in Diagram B, the trees in Trial C are shown in this way. There is very clear evidence that the trees on the rootstocks. in these five groups, in their first blossoming season, showed a marked decrease in productivity associated with increase in vigour even in the most dwarfing

groups. Thus, the trees on the rootstocks in Group 1, Trial C, produced at this age, on the average, 0·6 more trusses than did the trees in Group 2, while the latter produced five more trusses per tree than did those in Group 3.

Although it would be decidedly premature at this stage to attempt to draw any very definite conclusions as to the relative productivity of the rootstocks in this series compared with those in the Malling " Paradise " series, certain general tendencies have become sufficiently marked to deserve comment. Thus, although trees on all the eight rootstocks in this series, more dwarfing than

70 15 99 109 93 22 29 96 IX 108 9 116 90 VII 128 122 125 112 45 119 89
DIAGRAM B. NUMBER OF TRUSSES IN FIRST YEAR AVERAGE PER TREE

Malling No. IX, in the same trial, had borne a greater aggregate of trusses in their first season, by the end of their third year the trees on No. IX were more prolific than all except one of them. By the end of their sixth year, in Trial A, trees on the other two rootstocks in Group 3, and those on the four rootstocks in Group 5, had all borne a larger number of trusses than had the trees on No. IX, while those on the four rootstocks in Group 2 had borne fewer. This result is intimately bound up with relative tree size. Assessed on the basis of trusses produced per unit of new wood, eight of these rootstocks have been found to be more productive, up to their third year, than Malling No. IX.

The trees on Malling No. VII, growing in Trial C, bore fewer trusses than those of all, except one, of the rootstocks in the series. In Trial B, on the other hand, at the end of their fourth year, they were among the most prolific, having

borne considerably more trusses than those on Malling No. IX. As Table VI shows, however, on the basis of the trusses borne per unit of new wood, Malling No. VII was about equally productive with the other rootstocks in the same vigour-group at the end of its third and fourth years.

(d) As Measured by Fruit Production.
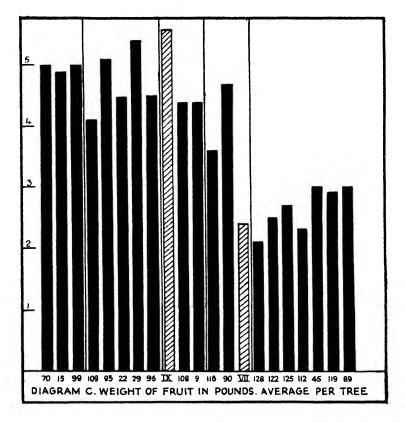
The fruits were counted and weighed each year. The total weights up to the end of the third, fourth and sixth years respectively are summarized in

TABLE VIII.

Average Total Crop per Tree in Pounds.

Rootstock No.	Trial A. Budded in 1930. Six years total.	Trial B. Budded in 1932. Four years total.	Trial C. Budded in 1933. Three years total.
Group 1.			
70	—	—	5·0
15	—	—	4·9
99	—	2·6	5·0
Average :	—	**2·6**	**5·0**
Group 2.			
109	15·2	—	4·1
93	30·1	—	5·1
22	23·2	—	4·5
29	13·2	2·8	5·4
96	—	—	4·5
Average :	**20·5**	**2·8**	**4·7**
Group 3.			
IX	20·6	2·7	5·6
108	21·6	2·8	4·4
9	20·3	2·5	4·4
Average :	**20·9**	**2·6**	**4·8**
Group 4.			
116	—	—	3·6
90	—	2·7	4·7
VII	—	1·2	2·4
Average :	—	**2·1**	**3·6**
Group 5.			
128	—	1·4	2·1
122	—	—	2·5
125	23·4	1·4	2·7
112	21·0	1·4	2·3
45	23·2	1·0	3·0
119	—	—	2·9
89	30·3	—	3·0
Average :	**24·4**	**1·4**	**2·6**

Table VIII and those for the rootstocks in Trial C are shown in Diagram C. The rootstocks are arranged in both cases in order of increasing vigour in the five vigour-groups. At the end of three years, the trees on Malling No. IX (in Trial C), had actually borne a greater weight of fruit, on the average, than had those on any of the rootstocks in the VIII x IX series. In general, however, and in spite of minor exceptions, there is a very close correlation between tree size and crop. Thus, the trees on the three rootstocks in Group 1 bore,

70 15 99 109 95 22 29 96 IX 108 9 116 90 VII 128 122 125 112 45 119 89

DIAGRAM C. WEIGHT OF FRUIT IN POUNDS. AVERAGE PER TREE

over the three years, a total of 5 lb. of fruit per tree, while, at the other extreme, the trees on the seven rootstocks in Group 5, bore approximately half this amount. Between these two extremes the trees on the rootstocks in the various groups can be arranged in a strikingly regular series. Among the four year old trees, in Trial B, this tendency can still be seen, although the differences between the trees on the rootstocks in the first three groups had to some extent disappeared.

Up to the end of their sixth year, the trees on the rootstocks in the vigorous group, in Trial A, had borne a somewhat greater weight of fruit than had the trees on the rootstocks in the less vigorous groups. This result is, of course, in line with that obtained with respect to blossom formation and arises from the same causes.

Seven of the ten rootstocks of the VIII × IX series, planted in Trial A, yielded a greater weight of fruit than did the trees on Malling No. IX, growing in the same trial, in their first six years.   Actually the trees on No. 93, slightly more vigorous in this particular trial and at this age than were the trees on the dwarfing Malling No. IX, produced, in the six years, almost 10 lb. more fruit per tree than did the trees on Malling No. IX.   In the vigorous group, the trees on No. 89 also produced, during this period, an average of almost 10 lb. more fruit than the trees on Malling No. IX.   Since the conditions under which the trees in this trial were growing were not as uniform as in the later trials, these results must not be regarded as more than an indication of the economic possibilities of this series.

It is interesting to notice the close association between the " dwarfing " character and " precocity ", so very marked in the rootstocks in this series. The fact that two such " precocious " rootstocks as Malling Nos. VIII and IX when crossed gave rise to seedlings not only apparently much more " precocious " but also much less " precocious " than themselves, is yet another indication of their very heterozygous constitution.

III. THE INFLUENCE OF THE ROOTSTOCK ON THE HEALTH OF THE SCION.

Since the casual incidence of outbreaks of diseases and pests might introduce a differential element into the trials, careful records were kept of their occurrence.

TABLE IX.

Relative Susceptibility to Leaf Scorch.   Average per Tree.

Rootstock No.	Relative severity of attack.*
45	0·275
9	0·425
128	0·425
90	0·425
112	0·475
125	0·475
29	0·625
IX	0·625
99	0·675
108	0·800
VII	1·700

* The trees were marked in order of increasing severity of scorching from 0 = no scorching to 10 = very severe scorching indeed.   The above figures are the averages of 50 trees in each case.   The brackets in Tables IX, XI and XII indicate that there was no statistically significant difference between the rootstocks within them.

Such data might provide evidence of a definite influence of the rootstock upon the resistance or susceptibility to disease of the scion and of the practicability or otherwise of breeding rootstocks capable of imparting resistance to or immunity from disease to the scion. The results set out in the Tables which follow were, in most cases, based on observations made during a single season and, while they illustrate the range of variation in relative susceptibility, they may be subject to modification in subsequent years.

TABLE X.

Number of Clean and Scabbed Fruits expressed as a Percentage of the Total Number of Fruits. Average per Tree.

Rootstock No.	Trial B.				Trial C.			
	Clean	Slight	Moderate.	Severe.	Clean	Slight.	Moderate.	Severe.
Group 1.								
70	—	—	—	—	64·1	24·3	7·1	4·5
15	—	—	—	—	76·2	17·2	4·8	1·8
99	80·6	13·4	4·8	1·2	61·5	22·9	13·3	2·3
Average :	**80·6**	**13·4**	**4·8**	**1·2**	**67·3**	**21·5**	**8·4**	**2·8**
Group 2.								
109	—	—	—	—	71·8	18·9	8·4	0·9
93	—	—	—	—	73·4	19·2	6·8	0·6
22	—	—	—	—	73·4	15·9	8·4	2·3
29	91·5	6·8	1·4	0·3	67·7	22·8	8·0	1·5
96	—	—	—	—	64·6	24·6	9·2	1·6
Average :	**91·5**	**6·8**	**1·4**	**0·3**	**70·2**	**20·3**	**8·1**	**1·4**
Group 3.								
IX	89·2	8·2	2·3	0·4	68·5	20·0	9·6	1·9
108	85·0	10·9	2·2	1·9	72·9	20·9	4·9	1·3
9	83·8	12·3	3·2	0·7	69·9	20·0	8·3	1·8
Average :	**86·0**	**10·5**	**2·5**	**1·0**	**70·4**	**20·3**	**7·6**	**1·7**
Group 4.								
116	—	—	—	—	72·4	21·2	5·0	1·4
90	91·0	5·0	3·8	0·3	79·0	13·5	6·2	1·3
VII	82·7	11·4	5·3	0·6	78·0	15·1	6·2	0·7
Average :	**86·8**	**8·2**	**4·5**	**0·5**	**76·5**	**16·6**	**5·8**	**1·1**
Group 5.								
128	91·4	4·7	1·8	2·1	85·0	9·8	5·2	0
122	—	—	—	—	83·9	11·1	4·5	0·5
125	96·4	2·8	0·4	0·4	79·2	13·8	6·3	0·7
112	94·2	5·8	0	0	74·4	17·9	6·8	0·9
45	96·2	1·7	1·1	1·0	82·3	13·5	3·3	0·9
119	—	—	—	—	74·6	17·9	6·6	0·9
89	—	—	—	—	71·4	20·2	6·8	1·6
Average :	**94·6**	**3·7**	**0·8**	**0·9**	**78·7**	**14·9**	**5·6**	**0·8**

(a) Leaf Scorch.

During the summer of 1936, the trees in Trial B showed considerable scorching of the leaves, and a record was made of its relative intensity. The records are summarized in Table IX. The trees were given marks from 0 to 10 according to the severity of the trouble. Those on No. 108, the most severely affected, were about three times as badly scorched as were the trees on No. 45, the least severely affected. The trees on Malling No. VII were more than twice as severely scorched as were the trees on No. 108. A statistical examination of the records suggests that the trees on some of these rootstocks may be markedly more resistant to this trouble than those on others. This has already been proved in the Malling series of " Paradise " stocks.

(b) Apple Scab.

The fruits obtained from the trees in the three trials were graded according to the severity of attack by Scab during 1936. The results for Trials B and C are summarized in Table X. The rootstocks have been arranged in order, of increasing vigour in the five groups and, as the Table shows, there was a very distinct correlation, in the two trials, between the vigour of the trees and the relative amount of Scab on the fruits. Thus, in Trial C, while the three rootstocks in Group 1 bore only 67·3% of clean fruit, the percentage of clean fruit on the trees on the seven rootstocks in the vigorous Group 5 was about 79. Between these extremes there was a gradual but fairly regular increase in the proportion of clean fruit with increasing vigour of the rootstock. This result is equally pronounced in the trees in Trial B, but was less obvious in the older trees in Trial A, probably because these trees, not being grown in randomized blocks, were more susceptible to positional influences. The causes of this very striking relation between increase of vigour and decrease of Scab-infection are rather obscure, but there is little evidence that any of these rootstocks are likely to be capable of imparting to the scion outstanding resistance to Scab.

(c) Apple Canker.

There was a fairly severe attack of Canker on the trees in Trial C during 1936, and records were made of its severity. In Table XI are shown the number of cankered shoots as a percentage of the total number of shoots on the tree. The average of forty trees is given for each rootstock. The trees on these rootstocks showed a wide range of variation in their susceptibility to this disease. Thus, the trees on No. 15 were, on the average, almost twenty times as severely cankered as were the trees on No. 112. These results suggest the possibility that the rootstock was exerting a differential effect on the scion, but they may possibly be attributable to other causes.

TABLE XI.

Number of Cankered Shoots expressed as a Percentage of the Total Number of Shoots. Average per Tree.

Rootstock No.	Percentage number of Cankered Shoots.
112	0·234
119	0·878
109	1·113
89	1·153
9	1·178
108	1·228
125	1·316
122	1·431
22	1·444
116	1·513
VII	2·147
45	2·319
99	2·350
IX	2·544
29	2·581
15	4·044

(d) APPLE MILDEW.

Attacks of Mildew were of frequent occurrence in these trials. It was relatively more serious, however, on the trees in Trial B, which had never made so much growth as had the trees in A and C. The severity of attack was recorded during 1936 and the results are summarized in Table XII. The number of shoots on each tree actually mildewed were calculated as a percentage

TABLE XII.

Number of Mildewed Shoots expressed as a Percentage of the Total Number of Shoots. Average per Tree.

Rootstock No.	Percentage number of mildewed shoots.
90	2·46
125	3·67
99	4·19
112	4·49
108	4·82
45	4·90
IX	5·49
9	5·52
128	7·35
29	7·74
VII	9·64

of the total number of shoots on the tree. Although the trees on No. 29 were actually about three times as severely affected as were those on No. 90, there was no statistically significant difference between the trees on the nine VIII × IX rootstocks shown in the Table. Such differences as were found were presumably due to chance.

(e) CROWN GALL.

During extensive tree excavations in the winter of 1936, the incidence of Crown Gall on the roots of the trees in Trial B were recorded. The trees on No. 108 had large galls in almost every instance, but they were not found to any marked extent on any of the other rootstocks. It may be added that " burr knots " were of frequent occurrence on the stock-stem portion of the trees on No. 112. So far as is known, there is no connection whatever between Crown Gall and " burr knots ".

(f) APPLE BLOSSOM WEEVIL.

An exceptionally severe infestation of Blossom Weevil occurred in Trial C during the spring of 1936. The " capped " blossoms were counted and the results are summarized in Table XIII. The figures in the Table are based on an average of forty trees on each rootstock and the number of " capped " blossoms is expressed as a percentage of the total number of blossoms on the tree. The rootstocks have been arranged in order of increasing vigour in the five groups.

The trees on the more vigorous rootstocks were relatively more severely attacked than those on the more dwarfing rootstocks. There was a fairly regular gradation in severity of infestation between the trees on the most dwarfing rootstocks in Group 1 and those on the most vigorous rootstocks in Group 5. This result appeared to be quite definitely related to the time at which the trees on the different rootstocks opened their blossoms. It had always been observed in these trials that the trees on the more dwarfing rootstocks were in full blossom some considerable time before the trees on the more vigorous rootstocks. It is thought that the most severe infestation of Weevils occurred, in this particular year, after the blossoms on the trees on the more dwarfing rootstocks were open, and while those on the trees on the more vigorous rootstocks were still in bud.

It may be said that so far as the limited observations on these trees have gone, little definite evidence of a direct influence of the rootstock on the resistance to disease of the scion has been obtained. In two instances, those of Scab and Blossom Weevil, a direct correlation between the size of the tree and

Table XIII.

Number of " Capped " Blossoms expressed as a Percentage of the Total Number of Blossoms. Average per Tree.

Rootstock No.	Percentage number of " Capped " Blossoms.
Group 1.	
70	9·2
15	8·1
Average :	**8·6**
Group 2.	
109	3·0
93	8·2
22	9·6
29	5·5
96	9·1
Average :	**7·1**
Group 3.	
IX	12·9
108	12·7
9	15·9
Average :	**13·8**
Group 4.	
116	14·8
90	10·6
VII	22·0
Average :	**15·8**
Group 5.	
128	20·3
122	10·0
112	22·3
119	20·7
89	19·4
Average :	**18·5**

relative freedom from infestation has been established. In the former, increase in size of tree was associated with a progressive freedom from disease, while in the latter the larger trees were those most severely affected. Quite obviously the underlying cause in the two cases was quite different. With Leaf Scorch, Canker and Mildew, such results as have been obtained do not suggest that any of the rootstocks in this series were capable of imparting any very definite resistance to the particular scion used, although the rootstocks do appear to exert slight differential effects on the relative resistance of the scion to Leaf Scorch and Canker. Only with Crown Gall on the roots was it possible to find a clear instance of the susceptibility of one particular variety of rootstock.

THE MORPHOLOGY OF THE ROOT SYSTEMS.

Some interesting morphological differences, principally in the relative amount and position of the fibre on the root systems of trees growing on certain of these rootstocks, were noted when the trees in Trial B were excavated during the winter of 1936-7.   They are fully reported on elsewhere (9).

The writer's sincere thanks are due to Miss J. L. Edgar for much assistance from the statistical side and to all those members, past and present, of the recording staff at East Malling, without whose unstinted help his task would have been impossible.   Finally, he is indebted to Miss K. Cornford for the photographs.

SUMMARY.

This paper describes studies, extending over six years, of trees worked on nineteen selected rootstocks from a family, Malling No. VIII (French Paradise) × Malling No. IX (Jaune de Metz), raised at the John Innes Horticultural Institution in 1923.

It is shown that there was an extremely wide range of variation in vigour between the trees of Lane's Prince Albert worked upon these rootstocks, whether expressed in terms of the new wood made, increase in thickness of the stems or the ultimate weight of the tree.

It was found possible to classify the rootstocks into five " vigour " groups. The rootstocks in two of these were found to exert an even more dwarfing effect on the tree than did the dwarfing Malling No. IX, growing in the same trials. At the other extreme, seven of the rootstocks exerted a definitely more vigorous effect than Malling No. VII at three years old.

A very close relation is shown to exist between the vigour of the trees on these rootstocks and the relative size of the swelling at the union.

When expressed in terms of blossom production, very considerable differences were found in the relative productivity of the trees on the various rootstocks.   A very clear and consistent correlation was found to exist between the " dwarfing " character and the productivity of the trees, the trees on the more vigorous rootstocks being, in every case, less productive, in their earlier years, than those on the more dwarfing rootstocks.

Although, in regard to the actual number of blossom trusses produced by the end of the third year, there was little perceptible difference between the trees on the rootstocks in the three least vigorous groups, it was possible to

demonstrate the greater relative precocity of the trees on the most dwarfing rootstocks, on the basis of the blossom trusses produced per unit of new wood growth.

In the first year of flowering, the trees on the most dwarfing of the series bore a considerably larger number of blossom trusses than did the trees on Malling No. IX growing in the same trials.
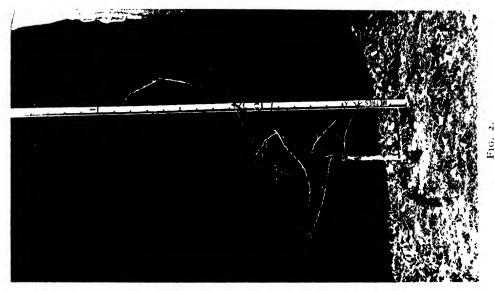
Records of fruit production by the trees on these rootstocks are summarized and discussed. It is shown that, in general, the conclusions that can be drawn approximate very closely to those already outlined for blossom truss production.
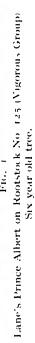
Data are given showing the relative susceptibility of the trees, during a single season, to Leaf Scorch, Scab, Canker, Mildew, Crown Gall and Blossom Weevil. Little evidence of a direct rootstock influence on the degree of attack on the scion could be deduced. A somewhat obscure correlation between the vigour of the tree and the relative freedom of its fruit from Scab is mentioned and an instance is given of the effect on the severity of apple Blossom Weevil attack on different rootstocks, due, probably, to the varying times at which the flowers on the trees growing on them opened.
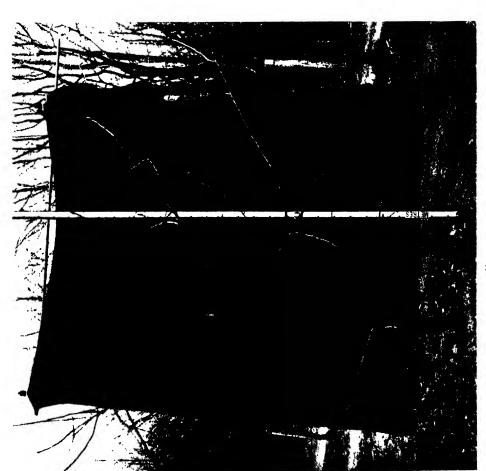
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Fig. 2.
Lane's Prince Albert on Rootstock No. 109
(Dwarfing Group).

Fig. 1
Lane's Prince Albert on Rootstock No. 125 (Vigorous Group)
Six year old tree.

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A CASE OF UNFRUITFULNESS IN BLACK CURRANTS

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I. INTRODUCTION.

The attention of the writers having been drawn to a black currant plantation in Belgium, in which almost the whole crop of fruit was said to " run off " each year, observations were made during 1934, 1935 and 1936 which showed that this was true of one particular variety, the flowers and young fruits, of which dropped to such an extent that the crop was a failure. This variety formed the greater part of the plantation, although there were also present in it a considerable number of bushes of Goliath, a few of Boskoop Giant and of some other undetermined varieties. The "running off" did not occur in Goliath (apart from the dropping of frozen flowers) but there were not enough bushes of Boskoop Giant to form an estimate. Of the remaining varieties some showed severe "running off", others set fruit excellently.

The variety in question was readily recognized as that known in Holland as Lee's Prolific, which was described and illustrated in the 1926-7 Report of the Experiment Garden at Breda (4). In Holland it was already known that this name was not the correct one for this variety, which, indeed, proved to be identical with one named Lee's Schwarze, introduced into Holland from a well-known nursery in Germany about 1920. The variety was compared with English material of Lee's Prolific, kindly supplied by Dr. Hatton, of East Malling, but was found to differ distinctly from that. Up to the present it has not been possible definitely to identify the variety ; in the present paper it will be referred to simply as Lee's.

The " running off ", or " coulure " as it is called in Belgium, was characterized by dropping of the fruit at an early stage, viz. a week or so after flowering. At that period the ground beneath the Lee's bushes was covered with fallen, unripe fruits and withered flowers. On some of the racemes no fruit at all was left, and dropping occurred equally from the terminal and basal parts of the racemes.

II. CAUSES OF UNFRUITFULNESS.

Premature shedding of fruits may be due to several causes. Crane and Lawrence (6, 7) have discussed generational and morphological sterility as well as incompatibility in this connection. Sometimes sterility may be due to fungus attack, as shown by Bennett (3) for wheat and by Dearness and Foster (9) for

loganberry. Insect attacks may also lead to barrenness. The virus disease of black currants known as Reversion is inimical to the setting of the fruit, and Ridler (16) showed that abnormal ovaries and ovules are associated with it. Hatton and Amos (12) pointed out that one of the symptoms of Reversion is the production of abnormal flowers. " Running off " of black currants was shown by Wellington, Hatton and Amos (19) to be due to the dispropor-tionate length of the styles, and consequent pollination failure. Finally, spring frosts and also high temperatures prevailing during the flowering period are injurious to the flowers and young fruits.

The investigations now to be described were undertaken to ascertain the cause underlying the failure of the Lee's black currant in the Belgian plantation to crop.

III. INVESTIGATION WORK.

(1) *Material used.*

Authentic cuttings of what will be designated as Lee's B, taken from the Belgian plantation in summer, were struck. They were grown in small pots during the winter preceding the spring in which the experiments were started. Similar material of Goliath was available and, in addition, cuttings of Lee's secured from two other plantations (here designated as Lee's Li and Lee's O) were used for purposes of comparison. Towards the end of February, 1935, the material was placed in a moderately warm glasshouse in the experi-mental garden at Breda, and the cuttings were gently forced into bloom towards the end of March. The pots stood plunged in a layer of peat dust, kept continuously moist, on the cement floor of the glasshouse. In the first season each cutting produced an average of three racemes of flowers. As soon as possible after ripening their fruit the cuttings were transferred to larger pots and placed out of doors, where they remained until the following spring. During the summer they made considerable growth, and they were kept in condition by watering with Wagner's solution (18). It was noted that the flower buds for the following season were borne on those parts of the shoots that had developed in the open air. In 1936 these plants each produced an average of about 100 flowers. They set their fruit much better than in the previous year, and better than other Lee's cuttings fruiting for the first time in 1936. Their set of fruit in the greenhouse was similar to that of full grown Lee's bushes (known to have been derived from the Belgian plantation) in the open air in a Dutch plantation near the Belgian border.

(2) *Crosses carried out.*

In trials carried on over four consecutive years, Wellington, Hatton and Amos (19) found that the four chief groups of black currants (Boskoop Giant,

French, Baldwin and Goliath) were self-compatible. They found, however, one variety only (Baldwin) that was unaffected by the exclusion of insect visitors. Lack of such visitors may lead to complete failure to fruit in certain varieties. As to Lee's, its tendency to " run off " and its failure to crop when insects are excluded have already been noted in Holland (4).

TABLE I.

Variety.	Pollinated with	No. of flowers pollinated.	No of fruits set.	% of fruits set.
Lee's B	Various Lee's	329	2	1
,,	Lee's B	23	0	0
,,	Lee's Li	56	0	0
,,	Lee's O	40	0	0
,,	Goliath	262	54	21
,,	Boskoop	40	3	7
,,	Roodknop	17	2	12
Lee's O	Various Lee's	115	0	0
,,	Lee's B	32	0	0
,,	Lee's Li	79	2	3
,,	Lee's O	30	1	3
,,	Goliath	62	12	19
,,	Boskoop	74	11	15
Lee's Li	Various Lee's	163	8	5
,,	Lee's B	20	0	0
,,	Lee's Li	36	0	0
,,	Lee's O	27	0	0
,,	Goliath	159	40	25
,,	Boskoop	45	14	31
,,	Roodknop	25	5	20
Goliath	Various Lee's	27	16	59
,,	Lee's B	69	33	48
,,	Lee's Li	33	9	27
,,	Lee's O	77	27	35
,,	Goliath	55	33	60
,,	Boskoop	39	13	33
,,	Roodknop	18	8	44
Boskoop	Lee's B	30	14	47
,,	Lee's Li	40	19	47
,,	Lee's O	53	20	38
,,	Goliath	6	3	50
,,	Boskoop	27	4	15
,,	Roodknop	11	0	0
Roodknop	Lee's B	24	14	58
,,	Lee's Li	14	3	21
,,	Lee's O	37	14	38
,,	Goliath	4	2	50
,,	Boskoop	26	17	65

To investigate this variety more fully, as large a number as possible of self- and cross-pollinations were made with it. Pollination was effected by removing a stamen with a pair of forceps from a recently opened flower and rubbing its anther against the viscid stigma of the flower to be pollinated. The pollen adhered well.

A start was made with the potted cuttings in the glasshouse in the spring of 1935, these being artificially pollinated. At the same time their own pollen was applied to the stigmas of flowers of other varieties. Often all the flowers of the different racemes on one plant were pollinated with pollen from a different variety, to serve as checks. The results of the crosses were judged by the number of ripe fruits produced and are expressed as percentages of the number of flowers to which pollen of a definite variety had been applied. They are assembled in Table I.

Summing up the relevant figures from this Table it will be found that Lee's, when selfed, set only 1% of fruit, whereas when crossed with Goliath it set 22%. Goliath, when selfed or crossed, set 48%. These figures are somewhat low when compared with those of larger plants, as will be seen later, but the difference between the selfed and crossed Lee's is very marked.   It is also clear from the Table that the self-sterility of Lee's cannot be due to incompetence of its pollen, for that pollen was satisfactory when used with other varieties. It might be suspected from the figures that Lee's pollen on Goliath was slightly inferior to Goliath pollen on Goliath, but the results obtained in the following year, 1936, did not support this view.

The crosses made in 1935 were repeated in 1936 in the glasshouse on some of the same Lee's cuttings as well as on certain others, and the results are given in Table II.

<div align="center">TABLE II.</div>

Variety.			Pollinated with		No. of flowers pollinated.		No. of fruits set.		% of fruits set.	
Lee's B	New cuttings	..	Lee's B	..	113		0		0	
,,	,,	..	*Lee's E.M.	..	82		8		10	
,,	,,	..	Goliath	..	459	652	58	87	13	12
,,	,,	..	Boskoop	..	107		18		17	
,,	,,	..	Baldwin	..	4		3		75	
Lee's B	Old cuttings	..	Goliath	..	261		104		40	
Lee's Li	,,	..	Lee's Li	..	435		0		0	
,,	,,	..	Goliath	..	489		210		43	
Lee's O	,,	..	Lee's O	..	394		2		0·5	
,,	,,	..	Goliath	..	519		162		31	

* Lee's Prolific, from East Malling, a different variety from the Belgian Lee's.

Here, again, it is seen that in the new cuttings Lee's, when selfed, produced no fruit. When crossed with Goliath the set was only 13% and the average with all foreign pollinations was 12%. The cuttings were probably not as well rooted as in 1935.   On one young Goliath cutting, however, 7 flowers pollinated with Lee's pollen produced 6 ripe fruits.   In the old cuttings the results are

still more striking and also more trustworthy, on account of the greater vigour of the plants and the larger number of flowers pollinated. They show clearly the necessity of foreign pollen for fruit production in Lee's (see Fig. 1). The quality of Lee's pollen was again tested by using it on Goliath, when 85% of ripe fruits were obtained, showing its competence.

Another series of pollinations was carried out in 1935 on bushes in the open in a mixed plantation of Lee's and Goliath. The branches used in the trials were encased in muslin ("Victoria lawn") sleeves, stretched on wire frames, just before the flowers began to open. As soon as opening started the flowers were emasculated. At least every second day the encased branches were examined, and thus the opening flowers were dealt with successively. Any flowers found in which the anthers had already dehisced were at once removed and eliminated from the experiment, thus avoiding unintentional self-pollination and the risk of possible antagonistic influence on the applied pollen, as found in cotton by Kearney and Harrison (14). The flowers were pollinated either on the same day as emasculation or on the following one by placing a small quantity of the required pollen on their stigmas.

In 1935, flowers of Lee's only were thus artificially pollinated. Three branches on different bushes were used for the Lee's pollen trial, and three others on the same bushes as well as one branch on each of three other bushes for Goliath pollen. Pollination was effected between May 1st and 8th, the coverings were removed on May 14th and the number of fruits was determined on June 28th, when they were already black. The results are given in Table III.

TABLE III.

Variety.	Pollinated with	No. of flowers pollinated.	No. of fruits set.	% of fruits set.
Lee's ..	Lee's ..	255	8	3
,, ..	Goliath ..	611	276	45

From this it is seen that the setting of fruit when Lee's is selfed is almost negligible as compared with that following crossing with Goliath.

These trials were repeated on the same bushes in 1936. Muslin was used for covering some of the branches, cellophane for others, whilst some branches were not encased at all. The results are given in Table IV and illustrated in Fig. 3.

Thus, selfing again produced very little set of fruit, whilst crossing with Goliath, whether under muslin or cellophane, resulted in a good set, better than in the previous year, possibly because the weather was cooler in May, 1936, than in 1935, when the first week of May was very hot and sunny. In the

TABLE IV.

Variety.	Pollinated with	No. of flowers pollinated.	No. of fruits set.	% of fruits set.
Lee's ..	Lee's (in muslin) ..	665	19	3
,, ..	Goliath (in muslin) ..	177	110	62
,, ..	Goliath (in cellophane) ..	146	89	61
,, ..	Goliath (in the open) ..	132	45	34
,, ..	(bee pollinated in the open)	1,330	624	46

relatively cold May of 1936 the muslin and cellophane coverings may have been an advantage ; this may explain why the set of flowers in the open crossed with Goliath was little more than half of that of the covered ones. On the whole, artificial pollination gave a better set of fruit than natural pollination by insects.

In 1937 the results of all pollinations confirmed those of 1935 and 1936. Lee's B, with its own pollen, set 0·1% of fruit from 951 flowers in the glass-house (checks 30% from 960 flowers) and 1% of fruit from 1,074 flowers under cover in the open (checks 40% from 694 flowers). In the plantation itself, artificial pollination with Goliath pollen gave a set of 53% of fruit from 377 unprotected flowers against 11% from 290 flowers following bee pollination on the same bushes.

In order to ascertain whether the Lee's and the Goliath pollen was vigorous, collections were made in Belgium and applied on the next day to emasculated flowers in Breda. The results were :—

Variety.	Pollinated with	No. of flowers pollinated.	No. of fruits set.	% of fruits set.
Goliath ..	Lee's ..	50	47	94
Lee's ..	Goliath ..	57	34	60

Thus, the pollen of Lee's from the Belgian plantation was perfectly efficient, and the Goliath pollen gave the same results as those obtained with it in previous trials. The reduced set obtained from Goliath pollen on Lee's must be looked on as due to some special character in Lee's ; it is certainly not due to faulty experimental methods.

The differences between selfed and crossed flowers are still more marked when the average weights of the fruits are compared. Thus, the average berry weight of Lee's selfed was 0·22 gm. in 1935, and 0·28 gm. in 1936 ; that of Lee's crossed with Goliath was 0·61 in 1935 and 0·99 in 1936. The berries of insect-pollinated Lee's averaged 0·36 gm. Flowers left to natural pollination

apparently suffered from a shortage of Goliath pollen and consequently did not produce large fruits.

Another experiment made in 1936 was with a single potted bush of Lee's. Some of the branches were exposed to insect visitors during the flowering period in immediate proximity to open Goliath flowers, others stood fairly near such flowers and still others a long distance from them. The pot was moved several times to obtain the necessary locations, and the branches exposed in one situation were encased when exposed in the two others. The results were as follows:—

TABLE V.

Situation of Plant.		Branches.	No. of flowers.	No. of fruits set.	% of fruits set.	
Close to Goliath	..	a	42	31	74	
Close to Goliath	..	b	11	7	64	
Fairly near Goliath	..	c	26	0	0	42
Fairly near Goliath	..	d	23	5	22	
Far from Goliath	..	e	72	4	6	
Far from Goliath	..	f	48	5	14	9
Far from Goliath	..	g	41	6	15	

The weather conditions during the experiment were not favourable to the visits of bees, but from the figures given it can be seen that when very near or comparatively so to Goliath, the set of fruits was about 42%, whilst at a great distance from this variety the set was only 9%, thus showing the importance of cross pollination in Lee's.

(3) *Similar behaviour in other Ribes Varieties.*

Amos (1) has reported " the almost total failure of the rogue types to set fruit ", but the cause of this does not yet appear to be clear. Further, according to Tydeman (17), " running off " is rather common in the seedlings of certain varieties. One variety of Ribes (" Groseillier sans pépin ") was already known to the present writers to exhibit very incomplete setting of the fruit in the experimental garden at Breda, and it was thought that the cause might be the same as that in Lee's. A single pollination experiment with this variety settled the question. From 750 selfed flowers only 131 fruits (17%) were obtained ; from 854 flowers crossed with the pollen of different varieties of red currant, 322 fruits (39%) resulted. This difference is sufficient to warrant the conclusion that foreign pollen is a remedy for the poor fruiting of " Groseillier sans pépin ". The similarity in behaviour to Lee's is more striking when the weights of the berries are considered. Selfed flowers yielded fruits averaging 0·44 gm. in weight, each containing a single seed ; crossed flowers gave berries averaging 0·81 gm., containing five seeds each.

(4) *Mycological aspects.*

It was thought possible that fungi might be responsible for the failure of Lee's to set fruit, one species (which was isolated and identified as *Cladosporium herbarum*) having been observed on the styles and between the pollen in the flowers of both Lee's and Goliath in the Belgian plantation in 1934. This fungus is usually regarded as a mere saprophyte, but spores of it were placed on the stigmas of flowers of both varieties a day or two before, just prior to, just after and a day after the application of Lee's pollen to both varieties, and Goliath to Lee's. With the former the number of fruits set in the presence of the spores was in both varieties slightly greater than in the controls to which no spores had been added. With Goliath pollen, the number was slightly less, but the difference was not significant enough to suggest any injurious effect from the presence of the fungus. In one instance only did the fungus make any headway. The inner surface of the calyx of a single Goliath flower was seen to be covered with it about ten days after inoculation ; nevertheless, this flower set an excellent fruit. It is not believed, therefore, that the " running off " of Lee's is due to attack by *C. herbarum*. As to other species of fungi, if present, disinfection trials were made to eliminate their possible effect. Before coming into flower the Lee's in the glasshouse were treated twice with Bordeaux Mixture, Lime Sulphur or Uspulun. After selfing, the Lee's again set no fruit, but when crossed with Goliath 39% of the flowers produced fruit. Disinfection therefore did not improve the setting of Lee's, and its failure is not believed to be due to any fungus attack.

(5) *Soil conditions.*

It is known that a dry soil induces fruit dropping, and water was therefore deliberately withheld from some of the plants in the glasshouse before the opening of the flowers. As a result the flower buds withered and dropped unopened, but this in no way resembled " running off ", i.e. the dropping of flowers and unripe fruits. Water was also withheld just after the fruit had set, but the fruits did not drop ; they ripened without reaching full size and remained attached to the stalks.

To ascertain whether nutrition was at fault, five plants were watered once or twice a week, starting before the flowering period and continuing for some weeks afterwards, with dilute Wagner's nutrient solution (18). They showed an improved leaf colour as compared with the untreated controls, but the selfed flowers still set no fruit, though those crossed set a rather larger number than the controls.

Soil acidity was also investigated. Two plants were grown in soil adjusted to a pH of 7 to 8, three others in one of pH 4 to 5. In both, the selfed flowers

set no fruits ; when crossed, 50% set in the alkaline and 42% in the acid soil. The figures do not point to any marked influence of soil reaction.

(6) *Reversion.*

This virus disease causes severe losses in yield. In the Belgian plantation very little Reversion was evident, but since it might have been latent there ten Lee's B scions were grafted in the spring of 1935 on two Goliath plants in pots, and ten healthy Goliath scions on Lee's B stocks. No symptoms appeared on any of the plants during 1935 or 1936, hence Reversion cannot be the cause of Lee's unfruitfulness.

(7) *Cytology.*

Since Crane and Lawrence (7) have shown that polyploidy is an important factor in causing sterility, the number of chromosomes was determined in Lee's, Lee's Prolific from East Malling, Goliath, Boskoop, Wellington, Naples Black, Daniel's September and Sivergieter's Black. In no case was the diploid number sixteen found to be exceeded. Polyploidy, therefore, is not the cause of fruit failing to set in Lee's.

(8) *Spring frosts.*

Undoubtedly spring frosts may cause serious damage to black currants, and the effects of this were carefully studied in the Belgian plantation. In 1935 damage occurred chiefly in Goliath, in 1936 in Lee's, which blooms somewhat earlier. Both varieties showed the same symptoms, viz. shrivelling and subsequent desiccation of the floral organs and shrinking of the ovary, the ovules drying up and becoming brown. The flowers afterwards dropped in a completely shrivelled condition, quite different from that seen in " running off ".

(9) *High temperature.*

High temperature during the flowering period sometimes causes " running off " in Ribes, but it is usually the terminal flowers on the racemes that open in the warmest weather, and fail to set fruit. In Lee's there is no such tendency, nor did temperatures somewhat higher than normal injure the flowers.

IV. MICROSCOPICAL INVESTIGATION.

As a result of the experimental work described above it was clear that the importance of foreign pollen for Lee's flowers was paramount above all other factors. Hence the processes of pollination and fertilization were subjected to microscopical study. The ovaries, in different stages of development, were fixed in Flemming's Solution and embedded in paraffin. Sections were cut and stained with either iron-haematoxylin or cotton blue. It was first thought that Lee's pollen tubes might not grow down Lee's styles, or at any rate too slowly to reach the ovules in time to effect fertilization. The tubes of

pollen from different sources were therefore studied in Lee's styles, and it was found that in both selfed and crossed flowers the tubes reached the ovaries on the second day after application to the stigma.

Following the lines indicated by Asami (2) and Yasuda (20) the influence of the ovaries was studied, and it was found that the presence of Lee's ovaries was of no consequence. In cut styles, whether from Lee's or Goliath flowers, the tubes made headway as rapidly when put on a Lee's as on a Goliath ovary or when left in moist air not in contact with any ovary. Thus, it may be concluded that the failure to set fruit in selfed Lee's is not due to any retarding influence on its pollen tubes exerted by its own stylar tissue or ovary.

Fertilization must be inhibited, therefore, at a later stage, viz. during the course of the tube through the ovary or at the moment of entering the micropyle. On the other hand, the embryo may die soon after fertilization has occurred.

Selfed and crossed flowers that had previously been emasculated were fixed at intervals after artificial pollination. Non-pollinated Lee's flowers were also fixed. It was thus possible to compare the course of events in normal setting with that ensuing when fertilization does not occur.

If "running off" is due to the failure of the pollen tubes to reach the ovule or of the male nuclei to fuse with the female ones, the sections would show no differences between the ovules in emasculated and non-pollinated Lee's or Goliath flowers and those in Lee's flowers artificially pollinated with Lee's pollen. If, on the other hand, fertilization apparently occurred, and a short-lived embryo was produced, the first stages after selfing would be similar to those following cross pollination, they would differ only later on, i.e. about fourteen days after pollination, when dropping was beginning. The results obtained were as follows:—

Emasculated Lee's flowers, not pollinated.—At the start the ovules appear quite normal, and the different nuclei in the embryo sac can be distinguished (Fig. 5). After 8 to 12 days the ovules begin to shrivel, the inner integument separates from the outer although the embryo sac is still normal ; at any rate the nuclei appear unchanged (Fig. 6). Beyond 12 days the embryo sac can no longer be distinguished, the contents of the ovule shrivel and degenerate (Fig. 11).

Emasculated Lee's flowers crossed with Goliath pollen.—A difference between the ovules that will develop into seeds and those that appear not to have been fertilized can be seen at least 5 days after pollination. The former increase considerably in size, the latter do not. After about 7 days these unfertilized ovules begin to shrivel, the inner integument separates from the outer, the volume of the embryo sac being thus diminished. The nuclei, however, remain visible for a few days longer. If an ample supply of Goliath pollen has been provided, these unfertilized ovules are soon pushed aside by

the swollen fertilized ones, the size of which has increased considerably by about 9 days after pollination. At this time, also, the embryo makes its appearance (Figs. 7 and 8). Later stages of embryos, about the 19th day, may be seen in Fig. 9. If only a very small number, such as five, of Goliath pollen grains have been applied, fewer ovules are pushed aside ; nevertheless these degenerate as described above. Hence it may be assumed that lack of pollen is the cause of degeneration in both cases.

Emasculated Lee's flowers selfed.—The development of the ovules in selfed Lee's is essentially the same as that in crossed flowers, but the number of ovules that swell is much less and they only very slightly push the unfertilized ones aside. The ovaries resemble those described above from flowers that received only very few Goliath pollen grains. (Compare Figs. 10 and 11.) The following figures for swollen ovules were obtained when ample supplies of both pollens were used :—

Days after pollination.	Goliath Pollen.	Lee's Pollen.
5	0	0
9	5–19	0–2
15	5–20	0–2
19	9–15	1–2 (3)

No " running off " occurred in this case until 9 days after pollination ; the minimum number of days before "running off " started in other experiments was usually 7. The time is probably dependent on temperature and prevailing wind. The following observations hold good for both selfed and crossed flowers of Lee's :—

(a) The embryo is visible in swollen ovules only ;

(b) A swollen ovule has never been observed to shrivel later on.

The critical moment must therefore lie before the ovule swells. Swollen ovules become seeds, and because an embryo can be detected only in swollen ovules it must be concluded that degeneration begins before an embryo is formed. In other words, the ovules shrivel because fertilization does not occur or the shrivelling begins immediately after nuclear fusion.

The microscopical preparations were compared with others made of " run off " flowers collected in the Belgian plantation in 1935 as well as from the experimental plants in that year and in 1936. The number of swollen ovules in " run off " fruits was, on the average, less than 1 (on a single occasion 3 were noted) whilst the fruits that remained attached until after June 6th contained an average of 12. The remainder had shrivelled in exactly the same way as in the experiments described above. The condition greatly resembles that of selfed flowers seven or more days after pollination. Such selfed flowers began to drop at least a week after pollination. Since the flowers in the plantation

were observed to drop about fourteen days after blooming, roughly speaking, the facts are concordant. The opinion that "running off" is due to lack of foreign pollen is thus confirmed by this comparison with selfed flowers. The conclusions to be drawn from the observations made are as follows: —

(1) Failure to fertilize is not due to insufficient growth in length of the pollen tubes.

(2) It is not connected with the formation of an embryo, which, being non-viable, afterwards degenerates.

(3) It may result from the failure of the pollen tube to penetrate into the ovule or the failure of the male and female nuclei to fuse.

Since neither of these stages has been met with in the preparations made, it has not been possible to determine the exact point at which the fertilizing process goes wrong.

V. OBSERVATIONS IN THE PLANTATION.

A careful watch was kept for any pests or diseases that might be responsible for defective fruit setting. In the spring of 1935 and of 1936 damage due to *Incurvaria capitella* was noted, but it was confined to the destruction of unopened winter buds. No other insects were noted. "Big bud" also occurred in the plantation, but its extent was not serious. Reversion was also seen, but chiefly in the varieties other than Lee's. The fungus *Stereum purpurem* was observed causing silvering of the leaves and killing many branches in the summer. On these dead branches its fructifications developed in the autumn.

In 1935 typical and severe "running off" occurred but it was less serious in 1936. This may be explained by the circumstance that in the latter year spring frosts injured Lee's to such a degree that the number of Lee's flowers remaining, in proportion to the quantity of Goliath pollen available, was more favourable than in the previous year. In order to check this supposition and to determine the approximate number of flowers and buds killed by frost, the original number of buds was counted on a few branches remote from each other and also the number of flowers that had opened or were likely to do so. In three out of four cases Lee's and Goliath branches were compared. The percentage of frost-killed flowers in Lee's was found to be 82, in Goliath 42. The percentage set of fruit of the undamaged flowers was 40 in Lee's and 75 in Goliath when examined on May 26th, 1936.

Wellington, Hatton and Amos (19) have pointed out that the visits of bees to black currant flowers are favoured by sunshine and shelter, and it was therefore expected that weather conditions, particularly wind and cloudiness, might be connected with "running off". Hence the attempt was made to follow the flowering and fruit setting on certain branches of Lee's by recording weekly the newly opened flowers and afterwards noting which of them had set fruit.

Unfortunately spring frosts interfered with the observations, hence the number of flowers recorded was much smaller than had been intended. Observations were also made on cloudiness and wind in the neighbourhood of the plantation during the period. The receptivity of the flowers was assumed to extend for both five and seven days. In both cases the results were the same, viz. the setting of the fruit was considerably better during the periods when the weather was favourable to bees—sunny or mainly sunny with slight wind only—than when it was unfavourable to them, and this supports the view that the " running off " in this plantation was due to lack of cross pollination.

The average berry weight and seed content was also determined for Lee's surrounded by and also remote from Goliath bushes. The average berry weight of the former was 0·73 gm. and the average number of seeds 19. In the latter, the berry weight was 0·57 gm. and the seeds numbered 9. In Goliath berries under similar conditions the average numbers of seeds were 23 and 25 respectively. From these results it is fair to conclude that the amount of available Goliath pollen was decisive for the fertilization of the Lee's ovules and the setting of the fruit.

SUMMARY AND CONCLUSIONS.

1. The variety of black currant known on the Continent as Lee's is not identical with the " Lee's Prolific " of English pomological literature. Its exact identity has not yet been established.

2. This variety is to a high degree self-unfruitful, and not very productive even when crossed.

3. Its pollen proved to be perfectly normal and viable ; its ovules also have a normal appearance.

4. Its unfruitfulness is manifested in the insignificant number of fruits set and their low individual weight, when not crossed by foreign pollen, e.g. that of Goliath. In the absence of such pollen the typical behaviour is a heavy drop of fruit within a week or so after flowering. In the present paper this is designated as " running off ".

5. One particular fungus (*Cladosporium herbarum*) was proved to have no connection with " running off ", and disinfection against the possible presence of others did not prevent it. The defect was not connected with soil factors (such as abnormal pH, insufficient nutrition and drought), Reversion, spring frosts or high temperature. Polyploidy was not detected.

6. The pollen tubes reached the ovary successfully, and degeneration of embryos did not occur. The cause of failure appeared to lie in the failure of the pollen tube to penetrate into the ovule or the failure of the male and female nuclei to fuse.

7. Observations made on a plantation in Belgium, mainly consisting of Lee's, taken in conjunction with the results of the investigations carried out, point to the necessity of ensuring adequate cross pollination by regularly and closely interplanting this variety with others (such as Goliath) if a reasonable set of fruit is to be secured.

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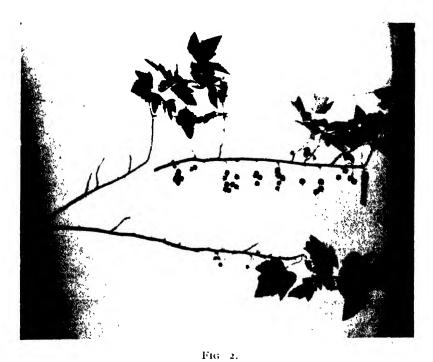
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FIG 1

Left plant   Ninety-seven " Lee's " flowers selfed , yielded no berries
Right plant   Ninety-eight " Lee's " flowers crossed with Goliath , yielded
thirty-three berries

FIG 2.

Top : " Lee's " branch, the flowers of which have been crossed with
Goliath   Bottom : Ditto ; flowers selfed.

FIG. 3

From top to bottom   Three " Lee's " branches crossed with Goliath
Fourth branch " Lee's " selfed   Fifth branch " Lee's " insect-pollinated

FIG. 4.

Pollen tubes reaching the ovary of a
selfed " Lee's " flower.

FIG. 5.

Unchanged embryo-sac in emasculated
" Lee's " flower.

FIG. 6

Inner integument retreating, eight
days after emasculating

FIG. 7

Ovary of crossed " Lee's " flower,
nine days after pollination

FIG. 8

Ovule of crossed " Lee's " flower,
nine days after pollination   Early
stage of embryo development
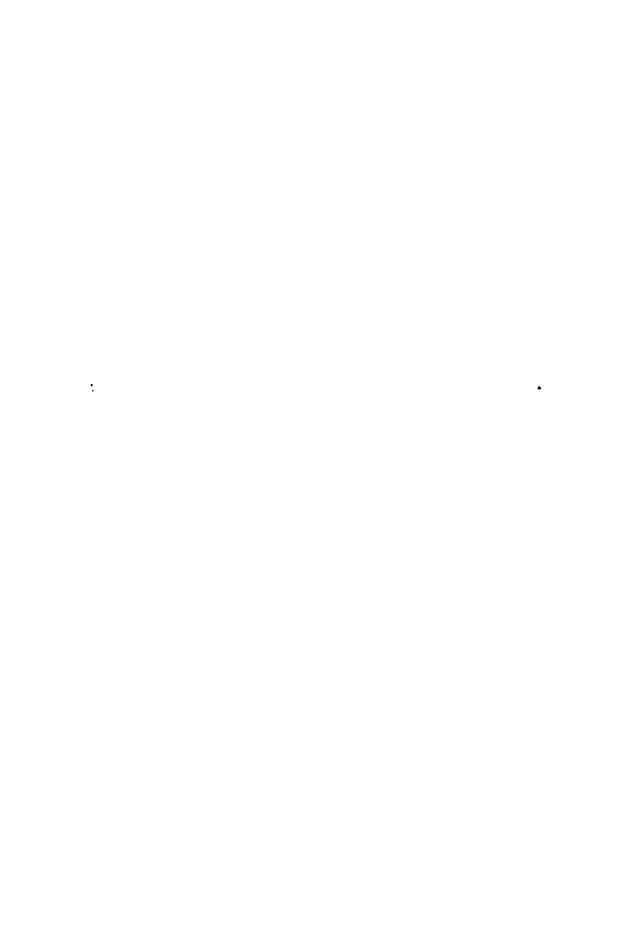
FIG. 9.

Embryo   development   nineteen
days after cross-pollination

FIG. 10

Shrivelled ovules pushed aside by
swollen ones thirteen days after
cross-pollinating " Lee's " flower

FIG. 11.

Shrivelled ovules thirteen days
after selfing.

OBSERVATIONS ON THE INCIDENCE OF DOWNY MILDEW ON NEW SEEDLING VARIETIES OF HOPS AT EAST MALLING, 1924-36

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INTRODUCTION.

A considerable part of the hop research carried out at the East Malling Research Station has consisted of the growing on, and observation of the behaviour of, new seedling varieties raised by Professor E. S. Salmon at the South-Eastern Agricultural College, Wye. Separate Reports by Professor Salmon on this work have been published annually by the Station.

The seedlings are grown in rows of from 15-18 hills each (a few in the earlier years, 30-32 hills) and are trained on the " Butcher " system with the top wire 13 ft. 6 in. high. The alleys are 7 ft. wide, and in about two-thirds of the garden the hills are 7 ft. apart in the rows, giving 889 hills per acre, whilst in the remaining portion the hills are 6 ft. apart in the rows, giving 1,037 hills per acre. The manurial and cultural operations have been uniform over the whole garden, but the type and quantity of manures applied have varied from year to year. Details of the manuring for each season are given in the Reports mentioned above.

Whilst the aroma of the best English varieties is very good, their preservative value is somewhat lower than that of the best Continental hops and very considerably below that of the choicest American sorts, which have a very strong aroma. The native American hop (*Humulus Americanus*) is distinct from the European hop (*Humulus Lupulus*) from which English and Continental varieties have been derived (1) hence seedlings raised from American hops crossed with English male hops are true hybrids. To obtain new varieties combining the good qualities of both, Professor Salmon has crossed Continental, American, and wild hops from New Mexico and Canada with English male hops, and the main object of the trials at East Malling has been to determine the relative cropping capacities of the seedlings and to provide material for chemical and brewing tests. However, the hop Downy Mildew (*Pseudoperonospora Humuli*) made its first appearance in this country in 1920 and spread rapidly from 1924 onwards, hence observations have been made on the incidence of this disease on the new seedlings.
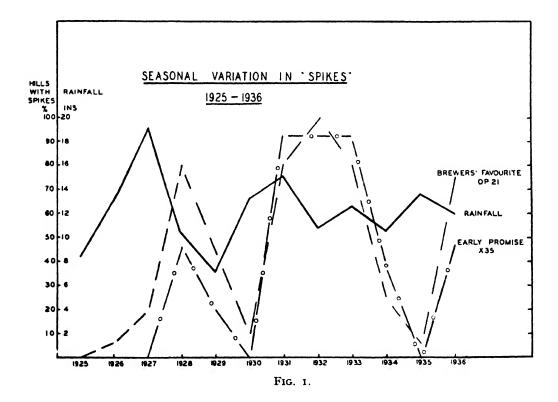
The disease has been described by Salmon and Ware (6) and it is not necessary to do more here than note briefly the various forms in which it

manifests itself. Early in the season stunted shoots known as " basal spikes " arise from the hills. On these, under damp conditions, spores are produced which cause infection of the leaves of normal bines and ultimately of the burr and cones. Moreover, during the course of the season the tips of the growing bines and the young laterals may be attacked and " terminal " and " lateral spikes " may arise. Finally, under certain conditions, the fungus may kill the bases of the bines during the dormant season, and in certain varieties, indeed, the entire rootstock. The course and intensity of Downy Mildew attacks during a given season is governed (apart from varietal resistance or susceptibility) almost entirely by weather conditions, being negligible or slight in a dry season, but devastating in a wet one unless the " spikes " are removed and the bine sprayed with Bordeaux mixture.

At East Malling, records have been taken at intervals throughout the growing season on the number of hills of each seedling showing spikes. Unfortunately it has not been possible to count the number removed from each hill, although those seedlings showing many spikes per hill have been noted. Thus, whilst the records give a reasonably accurate indication of those seedlings not prone to show this form of the disease, those with all hills showing a moderate number of spikes cannot be differentiated from those having all hills attacked, but with perhaps only one spike per hill. In certain seasons the cones have become attacked by picking time, and notes have been made on the presence or absence of cone infection of the seedling as a whole and not upon individual hills. In the earlier years all spikes were removed at frequent intervals during the season but no spraying was done. In more recent years the whole experimental garden has been sprayed with Bordeaux mixture (10 lb. Copper Sulphate, 15 lb. hydrated lime, 100 gals. water) as a rule twice during the season. This has largely prevented attack of the cones, and has resulted in healthy crops from most of the seedlings even in seasons favourable to the disease. In a mixed garden of some 300 very early to very late varieties, planted at random in small plots, it is naturally impossible to spray at the optimum time for each and every seedling. Those seedlings with only a moderate or slight amount of bine (particularly of " heads " over the top wire) at the time of spraying will be more copiously covered with the mixture than those having a heavy growth of bine. This difference in cover is greater when application is by " hop washer " than when hand lances are employed. Both methods have been used on different occasions. Differences in intensity of attack on the cones therefore, may not always be due to any real differences in susceptibility to infection, but to one or both of the above factors.

The collection of seedlings has undergone constant changes, those showing little merit after a few years having been grubbed and replaced by fresh selections. Very few have been in the garden over the whole period, 1924-36, so that for

many of them records of cone infection are not available over both sprayed and
unsprayed periods and for both good and bad seasons. Thus records of cone
infection can give only a very rough idea of the susceptibility of the seedlings.
Spikes arise in most seasons long before the first spraying (mid July) and the
records are therefore a fairly accurate measure of the tendency of the seedlings
to show the disease in this form. In some years (e.g. in 1936) lateral spikes
are formed late in the season, and burr and young cones may be attacked.
Such late infection will of course be influenced by spraying, as is also the
infection of the ripe cones.

SEASONAL VARIATION IN 'SPIKES'
1925 – 1936

FIG. 1.

SEASONAL VARIATION.

There has been very considerable seasonal variation both in the severity of
attack and in the form of infection most prevalent. The former is illustrated
in Fig. 1, which shows for two seedlings, Brewers' Favourite (OP21) and
Early Promise (X35), the percentage of hills* showing spikes in each season
from 1925-36. These seedlings have been growing in the garden over the
whole period, but others show a similar rise and fall in the severity of attack.

* Early Promise, 15 hills recorded through the whole period. Brewers' Favourite,
32 hills recorded 1925-30, 16 hills 1931-35, 140 hills 1936. "Brewer's Favourite" = (Oregon
Cluster × English Male) × English Male. "Early Promise" = Hop of unknown origin ×
English Male. Rainfall, April-September inclusive.

Particulars of the occurrence of the disease every year over the whole country have already been published (2, 3, 4, 5, 7, 8, 9, 10) but it did not appear at East Malling until 1924 and the main features of each season's attack there are as follows:—

1924. No basal, lateral or terminal spikes observed; attack on leaves of plants in nursery beds and on leaves and cones of some seedlings in hop garden.

1925. Both basal and terminal spikes occurred, reaching a maximum during July. Cones of some seedlings again attacked.

1926. A few spikes occurred, being most numerous during June. Certain seedlings again attacked on the cones.

1927. Spikes not very prevalent and reached their maximum by May 3rd. Many seedlings severely attacked on cones, some so badly that the crop was useless.

1928. Spikes reached a maximum at the end of July. Several seedlings were sprayed for the first time, but the cones picked from both sprayed and unsprayed were entirely free from the disease.

1929. Basal and terminal spikes not so common as in 1928, being most numerous about May 27th. Slight attack on the cones of the most susceptible seedlings.

1930. Spikes not so numerous as in two previous seasons, being commonest during the first half of June. Seedlings found susceptible to cone infection in earlier years sprayed on August 22nd. A number of seedlings, mainly unsprayed ones, were attacked on the cones.

1931. Spikes occurred very plentifully, reaching a maximum during June and early July. Two sprayings (July 15th and August 12th) protected the cones of all seedlings except those with an excessive amount of bine, many of which were attacked on the " heads ".

1932. Spikes extremely prevalent, a special feature being the large number of lateral spikes on the lower half of the bine at the end of June and beginning of July. Terminal spikes also numerous during June. The hops were sprayed twice (July 27th and August 10th) and the cones remained healthy in nearly all cases.

1933. Basal spikes not so prevalent as in the previous season. Terminal spikes in abundance, however, from May 24th to June 6th and at the latter date lateral spikes were also numerous on many seedlings. This was probably due to the fairly high rainfall (2·21 in.) during May. On June 19th an extremely severe hailstorm passed across the garden damaging all growing tips of bines and laterals and cutting the leaves to shreds. In consequence of this much new lateral growth was produced, and this proved very susceptible to attack, encouraged by the

heavy July rainfall (3·18 in.). Two sprayings (three for very late seedlings) were given and all the cones were picked free from disease.

1934. Few basal or terminal spikes. Bordeaux mixture was applied on July 18th and August 9th, and there was no attack on the cones.

1935. Spikes were rare and there was no infection of the cones. Two sprayings were given.

1936. Hardly any basal spikes, but the very wet weather at the end of June and during July caused a very serious outbreak of the disease during the burr period. Three sprayings were given over the whole garden, and the late seedlings received an additional application. With only a few exceptions, the crop was gathered free from disease.

INCIDENCE OF DOWNY MILDEW IN RELATION TO RAINFALL.

As the summer spores of the fungus require a film of water for their germination (2) a close correlation might be expected between rainfall and the severity of the disease, but it will be seen from Fig. 1 that there is little correlation between the severity of the disease on the two seedlings Brewers' Favourite and Early Promise and the total rainfall from April to September inclusive. There is, however, an apparent correlation between the rainfall of the previous summer and the development of spikes in the years 1926-31, though, with the exception of the season 1927-8, this is more apparent than real. This apparent lack of correlation can be explained with the aid of the monthly rainfall figures given in Table I. It will be noticed that there is great fluctuation in the rainfall from month to month, and that even in a wet season like 1927 some months may have a rainfall well below normal. Table I also includes the number of days on which rain was recorded in each month, since a large number of wet days, each with only slight rain, is likely to favour the spread of the disease more than a smaller number of wet days with a greater total rainfall. In most cases a high monthly rainfall is also associated with a large number of wet days, but there are certain exceptions. For example, September, 1933, had a high rainfall, but actually one day less on which rain fell than the same month in 1931, which had only about half the total rainfall.

In considering the incidence of the disease in relation to the rainfall, it will be more convenient to deal separately with the spike form of the disease and infection of the cones. Although spikes occurred in 1925 and 1926 they were only few in number. In 1927 they were more numerous, but not so plentiful as would be expected from the extremely heavy rainfall (over 19 in.) from April to September. It will be seen from Table I that April and May were very dry months and that the wet period did not begin until June. Evidently the fungus did not become active again in time to produce many spikes, but was able to cause a very severe attack on the cones.

TABLE I.

Summer Rainfall, 1924-36. East Malling.

No. of days on which rain fell.*

Month.	1924.	1925.	1926.	1927.	1928.	1929.	1930.	1931.	1932.	1933.	1934.	1935.	1936.
April	10	18	20	13	16	13	16	21	22	7	15	21	9
May	14	18	17	8	14	12	18	13	24	18	8	13	7
June	7	4	16	18	15	12	6	13	5	11	6	19	21
July	8	16	14	22	7	8	11	15	13	14	9	5	22
August	15	15	11	22	19	8	16	19	8	9	12	9	12
September	19	20	13	19	9	5	23	15	20	14	9	10	20
Total	73	91	91	102	80	58	90	96	92	73	59	87	91

Total Rainfall in inches.

Month.	1924.	1925.	1926.	1927.	1928.	1929.	1930.	1931.	1932.	1933.	1934.	1935.	1936.
April	3·31	2·01	4·77	1·58	2·01	1·17	1·28	3·94	1·81	0·85	2·87	3·31	1·31
May	2·21	2·01	1·86	0·84	2·00	1·34	2·32	1·99	3·06	2·21	0·71	1·82	0·44
June	2·03	0·28	2·64	3·73	2·40	0·98	0·50	1·32	0·69	2·01	1·48	1·63	3·95
July	2·99	0·35	1·71	3·96	1·58	1·04	2·16	2·41	2·12	3·18	2·02	0·94	2·80
August	1·91	1·79	1·32	4·57	2·25	2·19	3·64	3·42	1·26	0·69	1·83	2·67	1·20
September	3·75	1·80	0·67	4·76	0·34	0·61	3·30	1·94	1·97	3·56	1·38	3·57	2·34
Total	16·20	8·24	12·97	19·44	10·58	7·33	13·20	15·02	10·91	12·50	10·29	13·94	12·04

Month.	Mean Rainfall at East Malling 21 years.
April	2·14
May	1·66
June	1·53
July	2·21
August	2·14
September	2·14
Total	11·82

* Including those days when rain was observed, but the amount was too small to be measured (i.e. less than ·01 in.)

The great abundance of spikes in 1928, as shown in Fig. 1, can possibly be attributed to the extremely heavy rainfall during August and September in the previous year, and to the fact that no spraying was done in that season. On the view that heavy rainfall may wash numbers of spores to the ground and thus facilitate infection of the buds on the hills, an explanation is found for the great prevalence of spikes in 1928. Whilst the rainfall for 1928 would not account for the increase in the number of spikes over the previous season, the months of April, May and June were each slightly more rainy than normal and enabled the fungus to continue to spread, until checked by a dry July.

The number of spikes formed was less in both 1929 and 1930. The summer of the former year being extremely dry, the decrease was to be expected, but the summer rainfall of 1930 was above normal. This, however, came too late (August and September) to cause the formation of spikes.

It will be noticed that prevalence of spikes was very great in 1931-3 inclusive. Whilst both 1931 and 1933 had a rainfall above normal, that for 1932 was about an inch under the normal for the six months ; but in this year, May, the month when many shoots are coming from the hill, was exceptionally wet, whilst April had a large number of days on which rain fell. There was a large reduction in the numbers of spikes in 1934 and 1935, the reduction being greater in the latter season. The rainfall for 1934 was below normal, whilst in 1935, although April and September were wet, the intervening months were relatively dry.

In 1936 both April and May were dry and there were very few basal spikes ; June and July were rainy, however, and there was a great development of lateral spikes amongst the burr in the latter month.

As to the incidence of the disease on the cones, whilst the attack on those of certain seedlings in 1924 may be attributed to the heavy September rainfall, that of 1925 and 1926 must be considered as due to the susceptibility of the seedlings concerned and to the absence of spraying. As was to be expected from the very heavy rainfall in 1927 and the absence of spraying, the attack on the cones was extremely severe.

Cone infection was absent or only slight in the seasons 1928, 1929, 1932, 1933, 1934, 1935, 1936. Whilst the seasons 1928, 1929, 1932, 1934 were dry during August and September, in 1933 and 1935 September was wet, but the rainfall did not occur until towards the end of the month, so that the crop was picked before the fungus had time to re-establish itself after a quiescent period. In 1936 the absence of diseased cones can be attributed to a dry August followed by a September rainfall only just above normal.

The disease was present on the cones to a considerable extent on certain seedlings in 1930 and 1931. In the former year both August and September were wet, and in the latter, August only. From 1931 onwards the hop garden

has been sprayed with Bordeaux mixture at least twice each season, and this has prevented attack of the cones of all except the most susceptible seedlings.

It should be pointed out that many of the seedlings are extremely susceptible to cone infection and are likely to be attacked even in moderately dry seasons, unless sprayed. In unfavourable seasons they require more than two applications to secure a healthy crop. It is these very susceptible seedlings that have indicated the seasons mentioned above which favoured cone infection.

RELATIVE SUSCEPTIBILITY OF SEEDLINGS OF VARIOUS PARENTAGES.

The number of hills in the rows of the several seedlings has not been uniform, nor has the number of hills of any one seedling been constant from year to year. It is therefore necessary to express the number of hills showing spikes as a percentage of the total number of hills of the particular seedling in order to compare the figures obtained for the different seedlings. Owing to the fact that most of the seedlings have proved to be carriers of Mosaic, to which true Goldings and Golding varieties are highly susceptible, it has been necessary to eliminate the latter from the garden and also any seedlings that have proved susceptible to this disease. The only commercial variety in the Research Station garden for comparison with the new seedling varieties is Fuggle, which shows no symptoms of Mosaic and is considerably more resistant to Downy Mildew than Goldings and Golding varieties, which have proved to be, in wet seasons, very readily attacked on both bine and cone. In the earlier years, Goldings were grown, but the incidence of Mosaic rendered Downy Mildew records of little value.

Table II shows the incidence of bine and rootstock infection in the seedlings in the several parentage groups.

The figures are based on the severest attack on each seedling over the period during which it has been in the garden. In 1936 many seedlings showed a large number of lateral spikes amongst the burr, but as this occurred late in the season, it was probably influenced by the spraying which was then taking place ; it must be noted also that it took a certain time to record and remove these spikes, and a period of ten days elapsed between the making of records concerning the first and the last seedlings in the garden. The figures for 1936 have not therefore been used in compiling the Table. Seedlings planted out after 1931 have not been included in this survey, as the years 1934-6 have shown but little spike infection.

Late Bavarian × English male hop.

The late Bavarian variety was not named, but is believed to be allied to Hallertau, one which has proved to be very susceptible to Downy Mildew on the Continent. Out of a total of fourteen seedlings, eleven have proved highly

TABLE II.

Incidence of Bine and Rootstock Infection.

Parentage of Group.	No. in Group.	Number of Seedlings in each percentage grade of hills showing spikes.										Seedlings with hills killed.	Seedlings with strap-cuts only killed.
		91% to 100%	81% to 90%	71% to 80%	61% to 70%	51% to 60%	41% to 50%	31% to 40%	21% to 30%	11% to 20%	10% and under.		
Late Bavarian × English male	14	11	1	—	1	—	—	—	—	—	—	2	—
Late German Variety (260-1) × English male	17	12	1	3	1	1	—	—	—	—	—	—	—
German Variety "Elsass" × English male	4	3	3	1	1	1	1	—	—	—	—	—	—
(Saaz × English male) × English male	11	2	1	1	1	2	2	—	—	—	—	—	—
Seedlings of New Mexican Hop	27	26	1	—	—	—	1	—	—	—	—	—	—
Seedlings of Ygo (New Mexican Seedling)	8	5	—	2	—	—	—	—	—	—	—	—	—
Seedlings of Wild Hop, Manitoba	49	31	4	5	5	1	3	—	—	—	—	2	4
Oregon Cluster × English male	6	3	1	1	—	1	—	—	—	—	—	6	—
Seedlings of M45 (Oregon Cluster × English male)	31	9	7	5	3	3	2	—	1	1	—	14	2
Seedlings of OP13 (Seedling of M45)	25	11	4	1	3	3	—	1	1	1	1	11	—
(Oregon Cluster × English male) × English male, other than seedlings of M45	20	17	1	1	1	—	—	—	—	—	—	4	—
Tolhurst × English male	7	2	—	1	2	2	—	—	—	2	—	—	—
Seedlings of Golden Hop	16	6	4	2	1	—	—	1	—	—	—	—	—
Seedlings of Hops of Unknown Origin	14	7	2	2	3	1	1	—	—	—	1	—	—
Strains of Commercial Variety, Fuggle	12	—	—	—	—	—	1	4	3	—	2	—	—

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susceptible to attacks on the bine, with over 90% of the hills showing basal spikes in bad years. Three seedlings have been only slightly less severely attacked, having from 61% to 90% of the hills with spikes. These seedlings have, in certain seasons, shown a considerable amount of the angular spot form of the disease on the lower leaves. As well as having a large number of hills showing spikes, these seedlings have also had a large number of spikes per hill in seasons favourable to the disease.

Six seedlings have proved very susceptible to cone infection when not sprayed. Six of the eight seedlings that have not shown any cone infection have been in the garden only since routine spraying has been adopted. Two seedlings have suffered from infection of the rootstock and strap-cuts, but not to a serious extent.

Late German variety (260-1) × English male hop.

The late German variety (260-1) was imported for breeding work at Wye in 1902, and, owing to its richness in soft resins, was used as the female parent in a number of crosses. As the name of the variety is unknown, it is not possible to state its susceptibility to Downy Mildew when grown in its native land.

Out of seventeen seedlings, twelve have had over 90% of the hills showing spikes, whilst three have shown only slightly less severe an attack with over 70% of the hills affected ; two others have had an infection of between 50% and 70%. These seedlings are very similar to those of late Bavarian parentage as regards angular spotting on the lower leaves and the large number of spikes per hill.

Ten seedlings have been severely attacked on the cones when unsprayed. The remaining seven which have not shown infection of the cones, were not established in the garden before spraying was adopted.

German variety Elsass × English male.

Elsass is susceptible to Downy Mildew when grown in Germany. Only four seedlings have been under trial, and of these three have proved very susceptible to the spike form of the disease with over 90% of the hills attacked, whilst one has shown itself fairly resistant, with 41%-50% of the hills attacked. The three which produced most spikes have also proved susceptible to cone attack when unsprayed.

(Saaz × English male) × English male.

Prior to 1931, Saaz had shown itself to be practically immune from Downy Mildew in its native district in Czechoslovakia, but in that year its resistance broke down (4). In this respect its behaviour was similar to that of Fuggle in this country. Only one seedling (P13) of the first generation from Saaz has

been grown in the Research Station garden ; it has had 75% of the hills showing spikes and was badly attacked on the cones when not sprayed. However, eleven second generation seedlings of Saaz have been tested and these have shown a very even distribution as regards susceptibility to spikes, with from 41% to over 90% of the hills attacked.

Cone infection was recorded on nine seedlings in the earlier years before spraying was adopted. Neither of the two seedlings that have not shown cone infection was growing in the garden in the earlier years.

Seedlings of the New Mexican Hop (Humulus Americanus, *var. neo-mexicanus*).

All American cultivated varieties have proved very susceptible to Downy Mildew in their own country, but the reaction of *neo-mexicanus* to the fungus in its native country is unknown. Of a total of twenty-seven seedlings, twenty-six have had over 90% of the hills showing spikes, whilst the remaining seedling has had over 80%.

The seedlings appear to be particularly prone to produce lateral spikes on the first 5 ft. or so of the bine. Although the percentage of hills with spikes is high, the actual number of spikes per hill is usually decidedly less than that of seedlings of the late Bavarian and late German (260-1) crosses.

Fourteen seedlings have been attacked on the cones when unsprayed, and seven of these have shown cone infection even when sprayed. The thirteen seedlings that have not been attacked on the cones were established in the garden only after spraying became a routine measure.

Whilst the majority of these seedlings must be considered as highly susceptible to cone infection, it is doubtful if a few, such as " Cats-tails " (OZ79) and OI47, are much more susceptible than certain commercial varieties, such as Canterbury Golding. It is evident that clean crops of these seedlings can be obtained by prompt removal of all spikes combined with thorough spraying.

Seedlings of Y90 (Seedling of New Mexican Hop).

The seedling Y90 has proved very susceptible to infection of both bine and cones, the latter having been attacked when the hills were sprayed. Of eight seedlings so far tested, five have had over 90% of hills with spiked shoots, two have had 71-80%, and one has proved somewhat resistant to this form of the disease, with an infection of from 41-50%.

Two seedlings were attacked on the cones in 1931, a very wet summer, when only two sprayings were given. None of the seedlings was established in the garden when no spraying was done.

Seedlings of Wild Hop, Manitoba.

Of a total of forty-nine seedlings, thirty-one have proved very susceptible to infection of the bine, with over 90% of the hills bearing spiked shoots. The remainder showed a fairly even range of susceptibility with from 41% to 90% of the hills attacked.

Manitoba seedlings are liable to develop lateral spikes on the lower part of the bine, but to a rather less extent than the New Mexican seedlings.

Fifteen seedlings have been attacked on their cones, and thirteen of these even when sprayed. Of thirty-four seedlings that have not shown cone infection, ten were not established in the garden prior to spraying. Most of these seedlings make a very large amount of lateral growth late in the season, and therefore do not receive a thorough covering when sprayed.

The new variety, Brewers' Gold (C9a), of this parentage, which has in several seasons proved richer in preservative value than the best American hops, has, however, never been attacked on the cones at East Malling when sprayed. Two seedlings of this cross have had hills killed through the fungus entering the rootstock, and four seedlings have borne diseased strap-cuts.

Oregon Cluster × English male hop.

Only six seedlings have been under trial. Of these, three have had over 90% of the hills bearing spikes, and of the other three one has had an infection of over 80%, another of over 70% and the third of over 50%. One seedling has been severely attacked on the cones even after spraying, but the others have been free from any cone infection. Only one seedling was in the garden in the years before the adoption of spraying. All six seedlings have suffered severely from infection of the rootstock.

Seedlings of M45 (Oregon Cluster × English male).

The thirty-one seedlings have shown a fairly wide variation in the number of hills bearing spikes, but about half have had more than 80% of the hills attacked. Only two seedlings (OK14 and CC87) have shown considerable resistance, one with under 30% and the other with under 20% of spiked hills.

Prior to Bordeaux spraying, four seedlings were attacked on the cones. Of those that have not been attacked on the cones, four were not planted until after spraying was adopted. Out of the thirty-one seedlings, fourteen have had hills killed by the fungus, whilst two have had the strap-cuts killed. Included amongst those with hills killed is the seedling CC87 which suffered least (under 20%) from the spike form of the disease.

Seedlings of OP13 (Seedling of M45).

The twenty-five seedlings have shown a very similar range of susceptibility of the bine to that of the seedlings of M45. Three were not growing in the

garden in the years before spraying was adopted. Only one has shown cone infection, but this seedling (M35) is an early one and it is likely that spraying has normally been too late to protect the cones. The last application has often been made when M35 was in hop, and the immature cones perhaps already showing signs of infection. Hills of eleven seedlings have been killed by the fungus.

Seedlings of (Oregon Cluster × English male) × English male.

Included in this group are all the second generation of seedlings from Oregon Cluster, except those derived from M45 (Oregon Cluster × English male). Seedlings of this group have shown a greater incidence of spiked hills than those derived from M45. Of twenty seedlings, seventeen have had more than 90% of the hills showing spiked shoots, and none has had less than 60%.

Eleven seedlings have been attacked on the cones when not sprayed. Of the nine that have remained free from cone infection, three have been in the garden only since Bordeaux spraying became general.

Four seedlings have had hills killed by the disease, and three of these are seedlings of M52 (Oregon Cluster × English male).

Tolhurst × English male.

Tolhurst has proved very susceptible to Downy Mildew when grown in commercial gardens. Only seven seedlings raised from it have been under trial, and they have shown great differences in the incidence of the spike form of the disease. Two seedlings have had over 90% of hills showing spikes, three have had between 60% and 80%, whilst two seedlings have shown considerable resistance. One of these, L21, has had the very low figure of 12% of the hills attacked in a bad year, whilst during the four years 1933-6 inclusive, no spikes have been found on an experimental plot of about half an acre of this variety. The spikes from L21 have always been removed before they developed any spores. This seedling has also shown itself to be either immune from or highly resistant to attacks on the cones.

Three seedlings of Tolhurst parentage have, as might be expected, been attacked on the cones when not sprayed.

Seedlings of Golden Hop.

The Golden Hop is an ornamental variety with yellow leaves, probably of German origin, and is of no brewing value. It is, however, immune from attacks of the ordinary hop Mildew (*Sphaerotheca Humuli*), hence its use as a parent for new varieties.

Sixteen seedlings have been under trial, and of these six have had over 90% of hills showing spikes, a further six have had from 70% to 90%, three from 50% to 70%, and one between 30% and 40%.

No seedlings have been attacked on the cones, but none was planted in the gardens when spraying was not practised.

Seedlings of Hops of Unknown Origin.

These have been raised from several hops of unknown origin found growing in the Wye College gardens by Prof. Salmon when he began his breeding work. Of fourteen seedlings observed, half have had over 90% of hills bearing spikes, five have had from 60% to 80%, one has proved somewhat resistant, with between 40% and 50%, while one, P43, has shown very great resistance to the spike form of the disease, having had less than 10% of the hills affected.

Six seedlings (including P43) have been attacked on the cones when not sprayed. The infection of P43, however, was extremely slight and occurred only in the very wet season of 1927. It is interesting to note that the female parent (F1) of P43 was very prone to produce spikes when grown at the Station, as was also another seedling (X66) derived from it.

Fuggle.

Eleven strains of Fuggle* originally obtained from various growers in Kent, Worcester, Hereford and British Columbia, have been under trial. Whilst none of the strains has suffered severely from spikes, there has been considerable variation in the number of hills bearing spiked shoots in the several strains. The most severely affected strain (from Kent) has had between 60% and 70% of the hills attacked, whilst two strains (from Kent and Worcester) have had less than 10%.

Many experienced growers have always maintained that there are distinct strains of Fuggle in cultivation, and this view appears to be supported by the above behaviour. In addition, it may be mentioned that in a cultural trial at this Station, in which clonal Fuggle sets (originally obtained from three different sources in Kent) are being grown, one strain has proved more resistant to frost and has so far given heavier crops than the others.

Spores have never been noticed on the spikes collected from Fuggle hills at the Research Station. It must, however, be remembered that the spikes have always been removed very promptly, and spores would presumably have developed had the spikes been allowed to remain longer on the hills. The cones of Fuggle have never been attacked in the experimental garden, even when not sprayed. Prior to 1930 Fuggle, in commercial gardens, had appeared to be immune from attack on the cones, even in the severe outbreak of the disease in 1927. However, in 1930, cases were reported where Fuggle was severely attacked on the cones, and Salmon and Ware (3) considered that a possible explanation of this lack of resistance might be the occurrence of a new biologic

* Not of clonal origin.

form of the fungus capable of attacking Fuggle cones. The freedom from cone infection of the Research Station strains of Fuggle may, therefore, be due to the absence of this biologic form.

INFECTION OF ROOTSTOCKS AND STRAP-CUTS.

Diseased strap-cuts were first noticed at East Malling in the winter of 1928-9, on two seedlings. The cuts showed brown discoloured patches on the outside, and when cut open were found to be discoloured inside also. The dormant buds on affected strap-cuts are brown instead of a normal healthy white colour. It was not until the winter of 1931-2 that the presence of the disease in the strap-cuts and hills of commercial gardens was reported by Salmon and Ware, although hills in gardens in France were reported to have been killed in 1926 (5).

Since the winter of 1928-9 infected strap-cuts have been found on various seedlings at cutting time each season, although the severity of attack has varied from year to year. In many cases the mycelium of the fungus has penetrated and killed the rootstock.

Forty-one seedlings have had their strap-cuts infected and hills killed by the fungus. Of this number, no less than thirty-five are descended from Oregon Cluster. All six of the seedlings of Oregon Cluster × English male have been severely attacked in strap-cuts and hills. Fourteen out of thirty-one seedlings of M45 (Oregon Cluster × English male) have been attacked, and eleven out of twenty-five seedlings of OP13 (a seedling of M45) have shown also infection of rootstock and strap-cuts. Of twenty seedlings of the second generation from Oregon Cluster (excluding seedlings of M45) four have proved susceptible to rootstock infection, and three of these are derived from the seedling M52 (Oregon Cluster × English male). A few other seedlings have also been attacked, viz. :—

Seedling of Wild Hop, Manitoba (2).

Hop of unknown origin × male raised from Golden Hop (1).

Seedling of 59a (American seedling) (1).

Seedling of late Bavarian hop (2).

Of one seedling (OV7) of the late Bavarian hop, only a few hills and strap-cuts have been attacked. In some seasons a number of rooted sets of this and other susceptible seedlings raised in the nursery have been killed by the fungus. The normal practice at the Research Station is for the rooted sets to be lifted from the nursery early in November and then bedded in until required for planting, which usually takes place during February. The sets

may appear to be quite healthy when lifted, but at planting time many are often found to be badly diseased or completely killed. This form of attack was serious only in the winters of 1927-8 and 1936-7, both of which followed wet summers very favourable for the spread of the disease.

In the earlier years the bine in the nursery beds was allowed to trail over the ground, but since 1933 it has been trained on low wirework (4 ft.) and has been sprayed regularly with Bordeaux mixture, hence infection in the rootstock was not so severe in 1936-7 as in 1927-8.

Whilst the majority of seedlings that have proved susceptible to infection of both strap-cuts and rootstock, have also shown themselves to be very susceptible to bine and cone infection, there have been certain exceptions. For example, M45 (Oregon Cluster × English male) has proved so susceptible to infection of the rootstock that it has been found impossible to keep the plot fully planted up, and those hills that do not become completely killed are so weakened that the yield per acre of the variety has been reduced by half since the advent of Downy Mildew. This seedling is not, however, particularly subject to the spike form of the disease, and has never been attacked on the cones, even when unsprayed. A few seedlings have borne badly diseased strap-cuts, although the hills have remained healthy. Of these, four are seedlings of a wild hop from Manitoba. Brewers' Gold (C9a), a seedling of this parentage, has suffered so severely from diseased strap-cuts in some seasons that propagation has had to be by means of runners. However, the rootstock has remained alive and has given rise to sufficient bine to produce an average crop over eight years of over 25 cwt. per acre.

DISCUSSION.

Whilst the total amount of spike infection found in any year is largely determined by the seasonal weather conditions, the relative susceptibility of the various seedlings remains fairly constant, as is illustrated by the annual spike infection of Brewers' Favourite (OP21) and Early Promise (X35) shown in Fig. 1.

There are naturally some differences in susceptibility amongst seedlings of the same parentage, but seedlings of certain origins are notably more susceptible than others.

Most of the seedlings derived from late Bavarian, late German (260-1) and Elsass, together with all those of the New Mexican wild hop, are highly susceptible to attack on the bine, but the disease tends to give rise to rather different symptoms in the two groups. Seedlings derived from the first two German parents tend to have a large number of spikes per hill, and to

suffer from angular spotting of the lower leaves in years favourable to the fungus. The New Mexican seedlings have fewer spikes per hill, but have a definite tendency towards the production of lateral spikes on the lower half of the bine. Although so far only eight seedlings of the second generation from New Mexican Hop (seedlings of Y90) have been under trial, there is an indication that at least some of them will show less susceptibility than those of the first generation.

Whilst the great majority of the seedlings of the Manitoba wild hop are very subject to the spike form of the disease, a few are only moderately susceptible. The second generation of seedlings from Saaz and seedlings of Golden Hop range from very susceptible to moderately susceptible to bine infection, with a fairly uniform distribution.

The closest correlation between parentage and incidence of the disease occurs in seedlings derived from Oregon Cluster. All six seedlings of Oregon Cluster × English male have suffered severely from invasion of the rootstock by the fungus. This susceptibility has been passed on to the second and third generations via the seedlings M45 and M52 (seedlings of Oregon Cluster). Although many seedlings derived from M45 and OP13 (a seedling of M45) are liable to be killed by invasion of the rootstock, several seedlings show varying degrees of resistance to infection of the bine, even when attacked in the rootstock. Whilst seedlings of the second generation from Oregon Cluster, other than those of M45, are all very subject to the spike form of the disease, they are less liable to rootstock infection than those of M45 or OP13 (seedling of M45). Of twenty seedlings under trial only four have been attacked in the rootstock, and of these, three are derived from one particular Oregon seedling (M52), whereas nearly half the seedlings of M45 and OP13 have suffered from infection of the rootstock.

With the exception of certain seedlings derived from the New Mexican and Manitoba wild hops, all seedlings have produced cones free from disease when sprayed. It is therefore impossible to obtain an indication of the relative susceptibility of the majority of the seedlings to cone infection. It can, however, safely be assumed that those New Mexican and Manitoba seedlings which have been attacked on the cones even when sprayed, are highly susceptible to this form of the disease. Only two seedlings, L21 (Tolhurst × English male) and P43 (seedling of hop of unknown, English, origin) have shown marked resistance to the disease in all its forms. Unfortunately neither of these seedlings has proved of sufficient brewing value to warrant its introduction into commercial cultivation.

Under East Malling conditions, Fuggle has proved very resistant to the disease, but there are great differences between the several strains in the number of hills bearing spikes.

Six seedlings of various parentages have proved of sufficient brewing value and cropping capacity to warrant their introduction into commercial cultivation. They are the following:—

Brewers' Favourite (OP21) = (Oregon Cluster × English male) × English male.
Brewers' Gold (C9a)   = Seedling of wild hop, Manitoba.
Quality Hop (OO63)   = 3rd Generation from Oregon Cluster.
Fillpocket (Z62)   = 2nd Generation from Oregon Cluster.
Cats-Tails (OZ79)   = Seedling of New Mexican hop.
Early Promise (X35)   = Seedling of hop of unknown origin.

The percentage of hills showing spikes in each season from 1931-6 inclusive for these seedlings is shown in Table III. Owing to changes in the hop garden

TABLE III.

Percentage incidence of hills with spikes of certain seedlings of proved merit, 1931-6.

Variety.	1931	1932.	1933	1934.	1935.	1936.
Brewers' Favourite (OP21)	81	100	81	25	6	75
Brewers' Gold (C9a) ..	87	93	80	60	13	47
Quality Hop (OO63) ..	73	67	44	nil.	nil.	100
Fillpocket (Z62) ..	38	90	—	—	nil.	13
Cats-Tails (OZ79) ..	81	94	69	10	nil.	94
Early Promise (X35)	93	93	93	40	nil.	47

which necessitated the grubbing and replanting of Fillpocket, no records of the incidence of spikes on this variety are available for the years 1933 and 1934. The incidence of the disease in 1936 was almost entirely confined to lateral spikes amongst the burr, and the figures for that year are not therefore strictly comparable with the others which are based on the normal basal and lateral spikes formed earlier in the seasons.

It will be seen that whilst there is considerable seasonal variation, all varieties have shown a high percentage of hills with spikes in a bad year such as 1932. However, there seems some indication that the Quality Hop is rather less subject to basal spikes than are the others, although it was severely attacked on the fruiting laterals in 1936.

The varieties in question have shown greater variation in their susceptibility to attacks on the cones. Brewers' Favourite has proved to be the most susceptible of the six varieties. It was severely attacked in 1927, when it was not sprayed, and slightly attacked in 1932 when it was sprayed twice. This variety makes a large amount of lateral growth rather late in the season, and therefore requires either heavier or more numerous applications of spray in wet seasons. The most recent recommendations of Salmon and Ware (7) are that four applications should be given to commercial varieties, so as to

ensure protection in years favourable to the spread of the disease. It is likely that, given this treatment, the cones of Brewers' Favourite would be completely protected.

Although the season in 1936 was very favourable to infection of the cones, a clean crop of Brewers' Favourite was obtained with only three applications on the whole half-acre plot, with the exception of four rows. Owing to a defect in the machine, these four rows did not receive a good covering of Bordeaux mixture at the first spraying. This shows the importance of the early application in seasons favouring the disease.

Brewers' Gold always makes a very large amount of lateral growth, nevertheless it has been possible to grow a clean and heavy crop with normal spraying. It was not in the gardens when they were not sprayed, but in 1931, a year favourable to cone infection, two applications resulted in a crop free from disease.

Quality Hop has been in the garden only since spraying has been practised, and it has never been attacked on the cones.

Fillpocket, also, has never been attacked on the cones, even in 1930, when it was not sprayed.

The New Mexican seedling, Cats-Tails, has shown itself less susceptible to attack than another heavy cropping variety of the same parentage which had a trace of infection in 1932 and 1933. Both varieties have been sprayed, except in 1930, when neither showed any infestion of the cones.

Early Promise must be considered definitely resistant to cone infection, as it was in the gardens before the advent of Downy Mildew, has been there ever since and has only once been attacked on the cones. This attack, which was only slight, was in 1927, a bad year for the disease, and the variety was unsprayed.

ACKNOWLEDGMENTS.

SUMMARY.

An account is given of observations on the incidence of Downy Mildew (*Pseudoperonospora Humuli*) from its first appearance in 1924 until 1936 on certain new seedling varieties of hops and on strains of Fuggle growing in the experimental garden at the East Malling Research Station, the main features of each season's outbreak being mentioned briefly.

The seasonal variation both in intensity and in the form of the disease (mainly spike production and cone infection) is shown to be largely influenced by the spring and summer rainfall.

The incidence of the disease on seedlings of various parentages is recorded, and it is shown that seedlings of certain crosses have, on the whole, had a high percentage of hills bearing spikes. Certain seedlings of *Humulus Americanus* and its variety *neo-mexicanus* have proved very susceptible to attack on the cones.

Some crosses have given rise to very susceptible seedlings and also to others which show some resistance to infection of the bine.

The closest correlation between parentage and susceptibility to the disease is shown by rootstock infection, practically all seedlings severely attacked in this way having originated from the same American variety. Only two seedlings so far tested have shown any very marked resistance to the disease. Of six new varieties recommended for commercial cultivation, some show a measure of resistance to attack either of the bine (or rootstock) or of the cones.

At East Malling, Fuggle has proved very resistant. The data obtained lend support to the view that there are various strains of this variety in cultivation.

Attacks of the disease on the cones have largely been prevented by routine spraying with Bordeaux mixture.

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In addition to 2, 3, 4, 5 and 7 above, the general incidence of the disease year by year is given by Salmon and Ware in :—

(8) Journ. Min. Agric. (1925), **32**, 30-6 ; (1926), **33**, 149-61 ; (1927), **33**, 1108-21 ; (1928), **34**, 1093-99.

(9) Journ. Inst. of Brewing (1929), **35**, 20-5 ; (1930), **36**, 63-6 ; (1931), **37**, 24-31 ; (1932), **38**, 37-44.

(10) Journ. S.E. Agric. Coll. (1934), **34**, 107-118 ; (1935), **36**, 48-54 ; (1936), **38**, 48-52.

TEMPERATURE—COLD INJURY CURVES OF FRUIT

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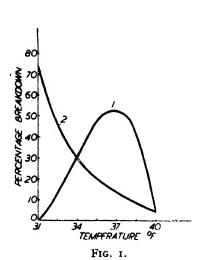
PART I.

GENERAL THERMOCHEMICAL CONSIDERATIONS.

STORAGE tests in South Africa have revealed a complex relation between the temperature of storage and the amount of physiological breakdown in various fruits. The results of these tests may briefly be summarized here, as a number of the original papers are still only in the course of publication.

Davies, Boyes, Beyers and De Villiers (10) observed that when samples of Japanese varieties of plums were stored at 31° F., 34° F., 37° F. and 40° F. for a period of twenty-five days, maximum internal breakdown occurred at 37° F. At 40° F. on the one hand and lower temperatures on the other there was less breakdown. Davies, Boyes and Beyers (9) later found that the maximum temperature at which breakdown of Japanese varieties of plums occurred was in the neighbourhood of 45° F. With lower temperatures breakdown generally reached a maximum at 37° F. and, with a storage period of twenty-five days, was reduced as the temperature fell below 37° F. These results have recently been confirmed by unpublished results of Davies and Boyes (5). The general result of all these experiments has been that breakdown usually reaches a maximum at an intermediate temperature around 37° F. and falls off either as the temperature is raised or lowered. An example is given in curve 1 of Fig. 1. Many other examples are given in the literature which has been cited. These results all refer to a relatively short storage period of about twenty-five days. With increasing periods of storage the position is gradually changed. This is a matter which will be discussed later—for the present only the results from relatively short periods of storage will be considered.

Pitting of the rind of Marsh grapefruit is similar. After three to four weeks storage pitting is rare both at temperatures as low as 30° F. to 32° F. or at temperatures as high as 50° F. to 55° F. In between these two extremes injury may be severe. This was first noticed in Florida (1) and, later, by Davies and Boyes (6). It has recently been confirmed with fruit from various parts of South Africa. A typical illustration is given in curve 2 of Fig. 2. This result, i.e. maximum pitting at an intermediate temperature, does not always hold for the more resistant, seeded varieties of grapefruit. These

FIG. 1.

The effect of temperature of storage on breakdown of two samples of Santa Rosa plums. Intermediate ("B") stage of maturity. Storage period of 25 days. After Davies, Boyes and Beyers (9).
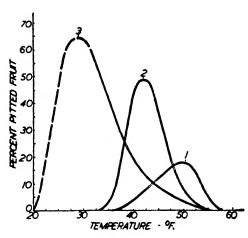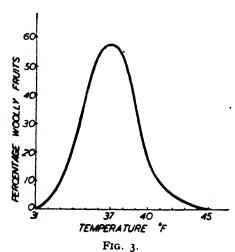
FIG. 2.

The effect of temperature of storage on pitting of Marsh grapefruit. Curve 1 from a very susceptible sample from the Western Province, stored for 14 days. Curve 2 from a sample from the Western Province stored for 23 days Curve 3 from a fairly resistant sample from the Eastern Province stored for 67 days.

FIG. 3.

The effect of temperature of storage on woolliness of Peregrine peaches. Intermediate ("B") stage of maturity. Storage for 24 days. After Davies and Boyes (8).
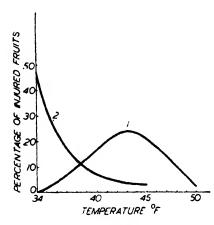
FIG. 4.

The effect of temperature of storage on injury to the rind of Navel oranges from the Western Province. Curve 1 from sample stored for 24 days. After van der Plank et al (18). Curve 2 from sample stored for 21 days. After Davies and Boyes (7).

varieties have generally been studied under conditions of prolonged storage for two or more months, a matter which is not being considered at this stage.

Woolliness of peaches behaves similarly. With a storage period of twenty-four days it is at a maximum at about 37° F. and is usually rare or absent either at 45° F. on the one side or 31° F. on the other (Davies and Boyes (8)). This is illustrated in Fig. 3.

Satisfactory relevant data for oranges are scarcer. Injury to the rind seems usually to increase regularly with decreasing temperature, giving the type of curve which has usually been accepted as a cold injury curve. This is shown by curve 2 of Fig. 4 which is taken from the results of Davies and Boyes (7). But on one occasion a peaked curve, similar to those already discussed, has been found. It is curve 1 of Fig. 4 which is constructed from the data of van der Plank, Rattray, Boyes and De Villiers (18).

The problem immediately arises whether these complaints which occur to a maximum extent at an intermediate temperature can be regarded as manifestation of cold injury. Whilst none of them occurs at high temperatures, yet all of them exhibit temperatures of maximum injury, below which injury decreases with decreasing temperatures. This would seem contrary to the generally accepted conception of cold injury. The objection appears superficial and unreal. It assumes that cold injury is due to a single factor, namely, the increasing maladjustment of the fruit to decreasing temperatures of storage. It ignores the fact that the actual visible manifestation of this maladjustment —i.e. the development of symptoms and lesions—involves processes within the fruit which are wholly or partly chemical in nature. The temperature relationships of cold injury are governed not only by the varying degrees of maladjustment of the fruit to varying temperatures, but also by the relationship between temperature and the rate of the chemical processes which finally render this maladjustment obvious. There are therefore at least two factors involved in a temperature-cold injury curve ; and it is with their interaction that this paper is concerned.

THE NECESSITY FOR A TEMPERATURE OF MAXIMUM INJURY.

A simple instance of cold injury is that of " tin pest ", i.e. the transformation in cold climates of the ordinary " white " form of elemental tin into the " grey " form which lacks the useful metallic properties which the element ordinarily possesses.* The change from one form into the other has been studied in some detail (see e.g. Mellor (14)). At temperatures above 18° C.—the transition temperature of one form into the other—white tin is stable, but

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* The authors wish to thank Professor R. B. Denison for calling attention to " tin pest " as an example of cold injury.

below 18° C. grey tin forms at a rate which increases with a fall of temperature until − 50° C. is reached. Below this temperature the rate of change reverses, and a further fall of temperature retards the transformation. There is thus a temperature of maximum rate of injury, corresponding with that found for breakdown of plums, woolliness of peaches and pitting of grapefruit. This is shown in Fig. 5.

The two factors which appear to be responsible for this curve are :—

(1) *An equilibrium factor.* When the temperature of the metal is below the transition temperature, 18° C., the white form is out of equilibrium ; and the degree of disequilibrium is related to the distance of the temperature from 18° C. There is probably no reversal in this process. The disequilibrium is probably always greater at a lower temperature ; and for this reason the transformation of white tin may be regarded as an example of cold injury in its strictest sense.
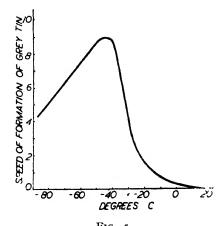
FIG. 5.

Relative speeds of transformation of ordinary tin to grey tin at different temperatures   After Mellor (14)

(2) *A kinetic factor.* For the disequilibrium at low temperatures to become manifest, there must be a chemical change from white to grey tin. This change, like any other chemical process, is subject to the thermochemical rule that the rate of change is retarded by a fall in temperature. This rule thus introduces a factor which operates with respect to changing temperature in the opposite direction to the equilibrium factor.

Both factors operate at all temperatures below 18° C. but their effects are not always equally apparent. At temperatures just below 18° C. it is the effect of the equilibrium factor which is the more obvious ; and if the temperature is brought up to 18° C. the transformation of white tin ceases because disequilibrium is eliminated. But at very low temperatures it is the effect of the kinetic factor which is the more obvious ; and if the temperature is brought sufficiently low the transformation will cease, as all chemical changes can be made to cease by a sufficient reduction of temperature. In between these two extremes there is a point at which the opposite temperature effects of the two factors are just balanced. This is the temperature of maximum rate of injury.

Cold injury to fruit appears to be similar. What constitutes a healthy equilibrium—or, in more conventional terms, a healthy metabolism—is not

known. Nor is it known what type of disequilibrium is responsible for the symptoms of cold injury.* But, whatever it may be, it must involve chemical changes ; and these changes must obey the rules of thermochemistry. The kinetic factor must therefore be admitted, and with it a temperature of maximum injury.†

Smith (15) has also suggested that two factors are present : a factor of " exposure " and one of " development ".

THE SPECIAL CASE OF A PEAK BELOW FREEZING POINT.

The general thermochemical considerations show the necessity for a temperature of maximum injury, but they do not specify what this temperature will be. In practice it is found that the temperature varies. It varies with the susceptibility of the sample and with the period of storage. Even for a single variety of fruit widely different values may be found. An example of this is given in Fig. 2, in which the temperatures of maximum injury of three samples of Marsh grapefruit are shown to vary by as much as 20° F.

The reason for this will become apparent later. At this stage it is desired only to point out that, to conform with the thermochemical considerations, maximum injury may occur anywhere on the absolute scale of temperature. It may occur on either side of the freezing point.

In the examples of peaked curves which have been considered, the temperature of maximum injury has been above the F.P. of the fruit, and has therefore been within the range of experimental demonstration. But when maximum injury occurs below F.P., no experimental demonstration of its presence is possible, because the purely extraneous phenomenon of freezing arbitrarily limits the range of temperatures at which fruit may satisfactorily be stored. Freezing injury masks ordinary cold injury ; and when the temperature of maximum injury falls below F.P. its presence can only be inferred.

An example of maximum injury below F.P. is given as curve 3 of Fig. 2. The continuous line was obtained experimentally for a rather resistant sample of Marsh grapefruit after relatively long storage. The broken line represents the expected continuation of this curve below F.P., which for grapefruit is in the region of 30° F. The whole curve shows a peak just below F.P. Curve 2

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* It is possible that the disequilibrium results from the increased tendency towards exothermic reactions at the lower temperatures. This tendency must occur on thermodynamical grounds, particularly in those reactions, such as oxidation reactions, which involve a considerable exchange of heat. Even if one assumes that the tendency is not always followed by reactions *in vivo*, the tendency itself must be admitted. One must concede either that the tendency is followed as it would be by reactions *in vitro* or that the protoplasm, energized by respiration, must actively intervene to maintain the normal equilibrium. Either alternative would involve an abnormal metabolism which, at a sufficiently low temperature, might conceivably lead to injury.

† This argument in its simple form holds strictly only for what we shall term " primary susceptibility " ; but with some modification of detail a similar two-factor hypothesis is applicable to " secondary susceptibility ".

for plums in Fig. 1 is probably another example of a peak below F.P. This is unusual for Japanese varieties in South Africa. Similarly, curve 2 for oranges in Fig. 4 is probably another example.

On this basis the type of curve which shows a regular decrease of injury with increasing temperature and which, for this reason, has been considered as the normal type of cold injury curve is regarded simply as an incomplete fragment of a peaked curve extending below F.P. Curves with a peak and curves without an apparent peak are essentially similar, the only difference being that in the latter case injury is at a maximum below F.P. This is not a great difference since experience has shown that the position of the peak is labile and easily changed.

A lowering of the peak, with the resultant tendency to bring it below F.P., can occur for three separate reasons :—

(1) *A higher degree of resistance of the fruit.* It is evident that the greater the resistance of the fruit, the lower the temperatures which are necessary to cause injury. In other words, the greater the resistance, the greater is the tendency for cold injury curves to be situated farther down the temperature scale, with the consequent greater likelihood of a peak below F.P. (Quantitative evidence on this point is given later—Figs. 8 and 9.)

(2) *A longer period of storage.* As the period of storage is increased, so is the temperature of maximum injury decreased until it tends eventually to fall below F.P. This is discussed fully in Part II (see Fig 6 (a)).

A longer period of storage and a high degree of resistance are generally associated, for the reason that it is only with resistant types of fruit that long periods of storage are usually attempted. Since both long storage and great resistance tend to lower the temperature of maximum injury, it is to be expected that examples of incomplete curves with peaks below F.P. will be found more frequently with fruits which are relatively well adapted to cold storage. Conversely, high temperatures of maximum injury are likely to be found more often with those tropical and subtropical fruits which are easily injured by cold.

(3) *An increased rate of those chemical changes which render injury manifest as symptoms and lesions.* A faster rate of chemical change would have the same effect as a longer period of storage, in both cases the lowering of the temperature of maximum injury being due to a greater degree of progress in the chemical processes which render injury manifest.

A high rate of chemical change is partly responsible for the low temperature of maximum injury shown by the incomplete curve 2 of Fig. 1. The rate of manifestation of injury in this particular sample of plums was so fast that considerable injury occurred at 31° F. even after only twenty-five days. Usually with plums in South Africa, the rate is too slow for appreciable injury to occur in this time at 31° F.

A fast rate of chemical change in Marsh grapefruit seems to be associated with a high degree of maturity of the fruit or with prolonged delayed storage at high temperatures (van der Plank (17)).
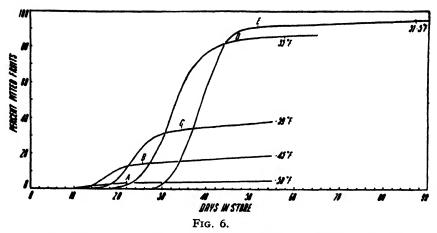
PART II.

PRIMARY SUSCEPTIBILITY WHICH IS INHERENT IN THE FRUIT AT THE TIME OF STORAGE.
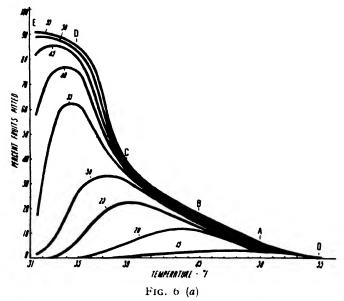
In Part I little attention was given to the effect of the length of the storage period. This has an important bearing on the question of cold injury curves and must be considered.

Fig. 6 shows the development of pitting in a sample of Marsh grapefruit from the Eastern Cape Province which was stored at six temperatures, 31·5° F., 35° F., 39° F., 45° F., 50° F. and 55° F. At 55° F. injury was negligible. At each of the other temperatures the behaviour is parallel. Injury begins after an interval of time and thereafter develops fairly rapidly. But it does not continue to develop until all fruits are affected, i.e. until 100% injury is recorded. Instead, it develops rapidly up to a point but beyond this it shows only a slight upward drift. These points are marked A, B, C, D and E on the 50° F., 45° F., 39° F., 35° F. and 31·5° F. curves respectively. For purposes of a first approximation, these points will be considered as true maxima throughout the whole of Part II of this paper. The slight upward drift beyond these points will be ignored for the present and discussed later (in Part III) as a separate phenomenon.

In another storage experiment, with Marsh grapefruit from the Eastern Transvaal, the absence of any great increase of pitting after the "maximum" had been reached was even more marked. Results are given in Tables I and II, Table I being for fruit in unwaxed sulphite wraps and Table II for fruit which had been wrapped at the packhouse in waxed crystalline paper. A part of the consignments was examined after five weeks and the rest after seven weeks. The Tables show no apparent increase of pitting during the interval, the small irregular change from five to seven weeks being well within the expected range of experimental error.

FIG. 6.

The effect of the period of storage on pitting of Marsh grapefruit
at different temperatures. Sample from the Eastern Province.

FIG. 6 (a)

The effect of temperature on pitting of Marsh grapefruit stored
for varying periods. The curves are numbered to indicate the
period of storage in days. From same data as for Fig 6.

A similar observation has been made on Marsh grapefruit from South
Africa by Tomkins and Dreyer (16). They found that although considerable
pitting resulted from cold storage during shipment to England, there was no
further increase if the fruit was re-stored after discharge. Similarly, Bates (2)
has recorded that cold injury to oranges in Rhodesia develops during the first
three or four weeks of storage at 36° F. and 40° F., and does not increase
greatly with further storage. A curve showing a flattening after maximum

TABLE I.

The Percentage of Pitted Fruits in a Consignment of Marsh Grapefruit from the Eastern Transvaal. Fruit in Unwaxed Sulphite Wraps.

Storage Period (weeks).	Storage Temperature.			
	39° F.	45° F.	50° F.	55° F.
5	73·1%	50·0%	16·0%	0·9%
7	68·0%	52·2%	11·3%	0·8%
Increase	—5·1%	2·2%	—4·7%	—0·1%

TABLE II.

This Corresponds with Table I, except that the Fruit was wrapped at the Packhouse in Waxed Crystalline Paper.

Storage Period (weeks).	Storage Temperature.			
	39° F.	45° F.	50° F.	55° F.
5	52·3%	13·8%	4·2%	0·2%
7	58·0%	14·2%	1·9%	0·0%
Increase	5·7%	0·4%	—2·3%	—0·2%

injury was reached has also been found in one instance for Kelsey plums stored at 45° F. (Fig. 7). Lastly, Bělehrádek (3, p. 93) working on haemolysis by cold of ox erythrocytes in the presence of digitonine obtained curves very similar to those of Fig. 6. In this case the maximum was a true one, and there was an entire absence of any drift in haemolysis beyond the maximum with an increase of time.
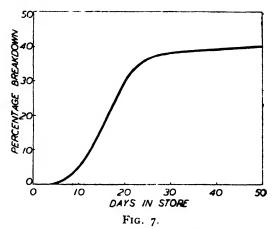
In showing a "maximum", the curves in Fig. 6 differ strikingly from the sigmoid "mortality curves" usually obtained from fruit in store.

The development of a maximum seems to admit of only one reasonable interpretation: When a sample of fruit is stored at a constant temperature, a portion of the sample, depending on the susceptibility of the fruit, is out of equilibrium from the very instant of cooling, and is predestined to subsequent injury. The rest of the sample is in a state of healthy equilibrium as far as cold injury processes are concerned—it is in this state of healthy equilibrium from the moment of storage and continues in this state indefinitely. Time itself does not bring about the disequilibrium of the susceptible portion; the disequilibrium is inherent in the fruit from the beginning. Time is necessary

only to complete the chemical processes which result from the disequilibrium and which eventually result in visible symptoms and lesions.

A clearer idea of the issue is obtained if one adopts from chemistry the conception of a transition temperature—i.e. a temperature above which fruit will remain healthy and below which it will be injured. On this basis one may consider that portion of the fruit which is predestined to injury as having a transition temperature above the storage temperature, with the consequent inevitable tendency towards transition to the injured state. Conversely, that portion which remains healthy indefinitely may be regarded as possessing a transition temperature below the temperature of storage and being consequently out of danger.

FIG. 7.

The effect of the period of storage on Kelsey plums stored at 45° F. After Davies, Boyes and Beyers (9).

Thus, to take a numerical example from Fig. 6, at 31·5° F. maximum injury was reached after about fifty-two days, at which time 90% of the fruits were injured. Further storage scarcely increased the proportion of injured fruits. The sample was thus made up of 90% of fruit with transition temperatures above 31·5° F. and 10% below ; and, at 31·5° F., the time necessary for the completion of the chemical changes which make disequilibrium manifest was about fifty-two days.

The term " primary susceptibility " will be used for this type of susceptibility which exists at the time of cooling.

THE TEMPERATURE COEFFICIENT OF COLD INJURY PROCESSFS.

At present there is no known method by which one can follow the course of the chemical process, or chain of processes, which results from disequilibrium with the storage temperature. The mortality curve is simply an indication of the variability of the biological material: i.e. the difference between the times at which the fruits show injury is a measure of variation between individual fruits in the sample. The actual chemical changes which eventually result in visible injury of any particular fruit are not reflected in the mortality curve. Nevertheless, one can at least determine the time needed for the reactions to run to completion in the sample of fruit taken in the mass. This is the time

taken to reach maximum injury. From this knowledge one may determine the temperature coefficient of the process.

From Fig. 6 it is seen that maximum injury was reached in about fifty-two days at $31\cdot5°$ F. ($-0\cdot3°$ C.) and in about twenty-five days at $45°$ F. ($7\cdot2°$ C.). Estimated from these figures, the value of Q_{10} within the range of the experiment is $2\cdot7$. In another experiment, also with Marsh grapefruit from the Eastern Cape Province, the corresponding times taken to reach maximum injury at $31\cdot5°$ F. and $45°$ F. were fifty-one days and twenty-three days. Whence the estimate of Q_{10} is $2\cdot9$.

These estimates are not very accurate because of the difficulty of getting a precise reading from the curves. Nevertheless, their accuracy is sufficient to show that Q_{10} has a value which is common for ordinary chemical processes. The evidence thus accords fairly well with what has been said about the kinetic factor in Part I.

With stone fruits, which will be discussed later, the temperature relationships are usually more complicated.

THE DEVELOPMENT AND PROPERTIES OF A PRIMARY

SUSCEPTIBILITY CURVE.

In Fig. 6 (a) are reproduced the same data as in Fig. 6, but they are now given in the form of a sequence of pitting-temperature curves at five-day intervals of storage, the curves being numbered to indicate the period of storage in days. The sequence begins in the region of the higher temperatures and gradually extends downwards to include the lower temperatures. In conformity with what has been said in Part I, the curves show a peak. At first this occurs at a high temperature. But with time it moves down to the lower temperatures until it eventually disappears beyond the range of the experiment, and the final curve is without a peak.

The sequence of curves in time builds up the curve O A B C D E from a temperature of $55°$ F. downwards. At $50°$ F. the point A, corresponding to point A in Fig. 6, is established after about twenty days ; and, considering matters as a first approximation, further storage causes no increase of pitting. Similarly at $45°$ F. point B is laid down in about twenty-five days ; at $39°$ F. point C in about thirty-five days ; and so on, until the whole curve O A B C D E is complete after fifty to fifty-five days.

The curve must be treated as an approximation—in reality it is a series of curves running close to one another. This is because the points A to E on Fig. 6 are not exact maxima ; the slight drift in pitting beyond the " maximum " results in a slight displacement with time of the curve in Fig. 6 (a). For present

purposes, however, this displacement will be ignored, just as the drift beyond the maximum in Fig. 6 has been ignored for purposes of approximation in Part II.

Considered in this way, the curve O A B C D E possesses a property which is unique among temperature-cold injury curves. When once sufficient time has elapsed for the curve to be formed, further time in store is without effect. The formation of the curve marks the elimination of the time element, and, with it, of the kinetic factor discussed in Part I.

By reading from the curves the amount of pitting corresponding to any temperature of storage, one obtains information on the primary susceptibility of the sample at any temperature. Thus, at $31 \cdot 5°$ F., $35°$ F., $39°$ F., 45 F., $50°$ F. and $55°$ F., the corresponding percentages of pitting are, roughly, 90%, 85%, 35%, 17%, 5% and 0% respectively. About 90% of the fruit therefore had a transition temperature above $31 \cdot 5°$ F. ; 85% above $35°$ F. ; 35% above $39°$ F. ; 17% above $45°$ F. ; 5% above $50°$ F. ; and none above $55°$ F.

Since this curve gives all the necessary information about the primary susceptibility, it will be called the " primary susceptibility curve ".
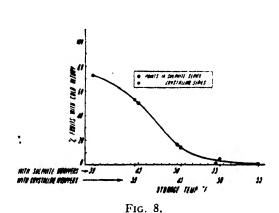
The properties of this curve are adequately defined if one knows (1) its shape and (2) its position relative to the temperature axis.

(1) *The shape of the curve.* This would seem to depend purely on the variability of the sample, i.e. on the difference in susceptibility between individual fruits in the sample. Curves which extend over a wide range of temperatures, as is usual with grapefruit, are indicative of great variability. It has, for example, already been pointed out that Fig. 6 (a) indicates that some fruits were so susceptible as to have a transition temperature above $50°$ F. while others in the same sample were so resistant that the transition temperature was below $31 \cdot 5°$ F. Steep curves, on the other hand, indicate a large measure of homogeneity. With plums, for example, the curves are often so steep that a few degrees suffice to bring about a change from o to 100% injury. That is, the transition temperatures of the individual fruits of the population fall within a very narrow range.

It is unnecessary to discuss the subject in detail. Suffice it to say that by subdividing the temperature scale at small finite intervals and by measuring the increase in injury over each of these intervals, one may classify the fruits in the sample according to the interval of temperature at which transition occurs. In this way an ordinary frequency curve may be constructed to show the distribution of the transition temperatures of the fruits within the sample.
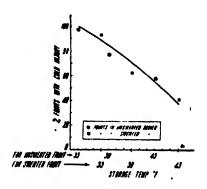
(2) *The position of the curve relative to the temperature axis.* It is evident that the position of the curve depends on the susceptibility of the fruit. The more susceptible the fruit, i.e. the higher the transition temperatures, the higher the curve will be situated along the temperature axis.

Experimental evidence indicates that the relation between susceptibility and position of the curve is very simple: Changing susceptibility is equivalent to the shifting of the whole curve, without modification of shape, up or down the temperature axis. This is shown in Fig. 8 which compares the pitting on Marsh grapefruit wrapped in unwaxed sulphite paper with that on fruit in waxed crystalline paper. The data are the same as those given in Tables I and II for a storage period of five weeks which, as shown by those Tables, is sufficient for maximum injury at all the temperatures concerned. In Fig. 8

FIG. 8.

A comparison of the effects of waxed crystalline and sulphite wraps on pitting of Marsh grapefruit at different temperatures. The comparison is made by an alteration of the temperature axis. The storage period was 35 days.

FIG. 9.

The effect of "sweating" for 6 days at 65° F. on the amount of pitting of Marsh grapefruit on subsequent storage for 45 days at different temperatures. The effect is shown in terms of a shift of the temperature axis.

the points for the crystalline-wrapped fruit are plotted against a temperature axis which is shifted 5.5° F. relative to the axis for fruit in plain sulphite paper. A single curve fits both sets of points. The effect of crystalline wraps in this experiment is thus seen to be equivalent to the raising of the storage temperature by 5.5° F. In other words, the increased resistance obtained by using crystalline wraps was equal to a reduction of 5.5° F. in the transition temperature of each fruit.

In Fig. 9 a similar comparison is made between "unsweated" Marsh grapefruit from the Western Province and fruit from the same source which had been "sweated" at a high humidity for six days at 65° F. The storage period was forty-five days, which was sufficient for maximum injury to be reached at all the temperatures concerned.

Two points must be observed with regard to the shifting of temperature-cold injury curves: Firstly, sufficient time in store must be given to allow the development of maximum injury, i.e. the curves must be fully-developed primary susceptibility curves. The results do not apply to the peaked curves

which are obtained with shorter periods in store. Secondly, the samples to be compared must be from the same source. They must be equally variable, i.e. the curves must be of the same shape, otherwise it is impossible to super-impose the one exactly on the other.

PART III.

SECONDARY SUSCEPTIBILITY, WHICH RESULTS FROM CHANGES IN THE FRUIT AFTER COOLING.

In Part II it has been considered that a maximum is reached, and that no further injury occurs after sufficient time has elapsed to bring about the injury of those fruits with a transition temperature above the storage temperature. This would be true if the transition temperatures existing at the time of cooling were fixed and unchangeable. Obviously, this is incorrect, although at times it may be sufficiently near the truth to serve as a good approximation. Fruit in store is not stable and immutable. It respires and continually ages* ; and as it ages, one may expect a change in the transition temperature, as one expects some changes in all properties of the fruit. If the tendency is for the transition temperature to rise, more fruit will be injured : and the gradual raising of the transition temperature will result in a gradual upward drift in the amount of injury.
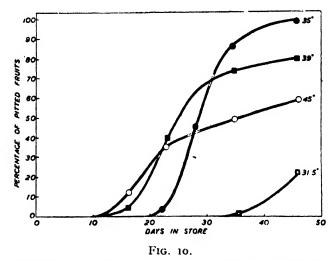
Such susceptibility which is developed *after cooling* by the ageing and weakening of the fruit in store will be called " secondary susceptibility ". Its presence may logically be deduced from the upward drifting of injury with time, just as primary susceptibility may logically be inferred from the development of maximum injury beyond which there is no drift.

The amount of secondary susceptibility which may be developed varies considerably. In the grapefruit from the Eastern Transvaal considered in connection with Tables I and II, it seemed to be absent, or very nearly so. All the injury developed within the first five weeks of storage, and further storage did not weaken the fruit. The grapefruit from the Eastern Cape, considered in connection with Fig. 6, was not quite so free from secondary susceptibilty. As the fruit aged in store, it showed some weakening, which resulted in a gradual upward drift of the " mortality curve " beyond the points hitherto considered, for the purpose of approximation, as maxima. Nevertheless, the injury due to secondary susceptibility was small in comparison with

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* Ageing, as referred to here, simply refers to the process of change with time in stored fruit. Up to the present there is insufficient evidence either to associate it with or to dissociate it from normal ripeness which occurs on the tree. For this reason the term " ageing " is used, because it is non-committal. It is hoped later to be able to compare or contrast " ageing " and normal ripening or senescence. But for the present, attention is being confined to the fact that there is an obvious ageing of fruit in store, without attempting to connect this ageing with any other process.

that due to primary susceptibility. But in all samples of Marsh grapefruit received from the Western Cape, the tendency towards secondary susceptibility was pronounced. The fruit weakened rapidly in store, with the result that the upward drift of the " mortality curve " was pronounced. Some examples will be considered later.

The reason for the presence or absence of a tendency towards secondary susceptibility is not yet known. (Some predisposing factors are at present under investigation.) It can be said at this stage merely that the factors which predispose a fruit to secondary susceptibility are not necessarily the same as those which cause primary susceptibility. This is illustrated by the sample of

FIG. 10.

The effect of the period of storage on pitting of Marsh grapefruit at different temperatures. Sample from the Western Province.
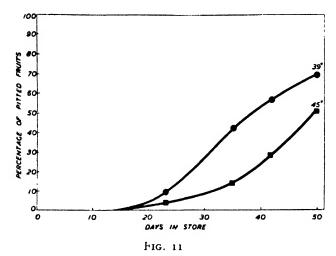
grapefruit from the Eastern Transvaal which has already been discussed. In this sample the primary susceptibility was great. This is shown by the high percentages in Table I. Yet, coupled with this high primary susceptibility, there was a virtual absence of any tendency towards secondary susceptibility.

A few examples of pronounced secondary susceptibility will now be considered.
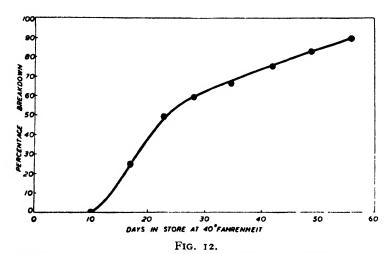
Fig. 10 shows the " mortality curves " obtained with a sample of fruit from the Western Province. Pronounced drifting is apparent at 39° F. and 45° F., and of the total pitting found after forty-five days a considerable proportion may be put down to secondary susceptibility.

Fig. 11 shows the " mortality curves " of another sample from the same orchard. With this sample the change from primary to secondary susceptibility is carried a step farther. Vestiges of the effects of primary susceptibility appear to exist in the " mortality curve " for 39° F., but at 45° F. no evidence of

primary susceptibility is apparent.   At the time of cooling, the transition temperatures of the fruit were probably below 45° F. ; and injury occurred only as the metabolic changes in store weakened the fruit and brought the transition temperature above 45° F.

FIG. 11

The effect of the period of storage on pitting of Marsh grape-
fruit at different temperatures   Sample from the Western
Province.

FIG. 12.

The effect of the period of storage on breakdown of Wickson plums
at 40° F.

As far as one can judge from the data in the literature (4, 11), most of the results with the more resistant varieties of grapefruit—or with relatively resistant samples of Marsh—indicate a similar absence of any marked effect of primary susceptibility.   Further work is needed on this point.
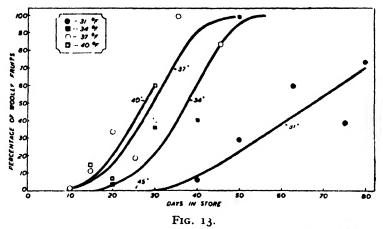
A pronounced tendency towards secondary susceptibility is shown in Fig. 12 for Wickson plums stored at 40° F.   The curve is similar to the 45° F.

curve in Fig. 10. Plums from the same orchard showed almost 100% break-down within twenty-five days at 37° F., which indicates that only a slight rise of transition temperatures is sufficient to cause secondary susceptibility at 40° F.

In one instance (Davies, Boyes and Beyers (9)—their Fig. 34) there is evidence of transition temperatures being raised above 50° F. with Wickson plums.

Apart from that given in Figs. 7 and 12, very little evidence on secondary susceptibility exists for stone fruits. As a general rule the primary transition temperatures of these fruits in South Africa fall within a narrow range, between about 40° F. and 45° F., the results in Figs. 7 and 12 being exceptions. It is therefore within this range that information on the balance between primary

FIG. 13.
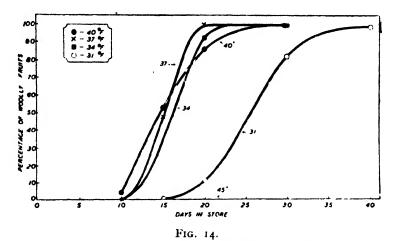
The effect of the period of storage on woolliness of Peregrine peaches stored at different temperatures. Fruit at 40° F. discarded for over-ripeness after 30 days. There was no woolliness at 45° F.
After Davies and Boyes (8).

and secondary susceptibilty is likely to be collected. Unfortunately, temperatures within this range have scarcely been studied.

The type of curve usually found for stone fruits is shown in Fig. 13 for Peregrine peaches and Fig. 14 for Elberta peaches. Results with plums are usually fairly similar ; examples will be found in the literature already cited. In Figs. 13 and 14 the rapid rise of woolliness at temperatures of 40° F. or below indicate high primary susceptibility. Secondary susceptibility may also be involved, but this is difficult to determine from the data.

Although they have no direct bearing on the immediate problem under discussion, there are two points about Figs. 13 and 14 which may be mentioned in passing. Firstly, the rate of development of woolliness at 31° F. is very slow relative to that at the other temperatures. The temperature coefficient of the rate of manifestation of injury is itself a function of temperature, and increases markedly as very low temperatures are approached. In this respect peaches

differ from grapefruit, which has already been discussed. (For instances of the inconstancy of Q_{10} for biological process see, for example, Bělehrádek (3).) It is this fact which allows very low temperatures to be used occasionally to avoid injury. Unfortunately, this is not invariably true of stone fruits—Santa Rosa plums, for example, have shown great inconsistency on this point. Secondly, the rate of development of woolliness at all temperatures was greater in the sample of Elbertas than in the sample of Peregrines. Consequently, with a storage period of twenty-four days, woolliness in Elbertas could not be escaped by storing at 31° F., in spite of the fact that the Elbertas, like the Peregrines, showed a marked increase of the temperature coefficient at the lower ranges of temperature. Fortunately, Elbertas ripen comparatively slowly in

FIG. 14.

The effect of the period of storage on woolliness of Elberta peaches at different temperatures. Woolliness was absent at 45° F. After Davies and Boyes (8).

store, so it is probably feasible to avoid injury without incurring over-ripeness by shipping at temperatures above the woolliness range. With Peregrines, which ripen more quickly but develop injury more slowly than Elbertas, it would probably be more satisfactory to ship at temperatures below the woolliness range.

AN ANALYSIS OF COLD INJURY—TIME CURVES.

The primary susceptibility, existing at the time of storage, takes into account the factors which affect the susceptibility of fruit up to the time of cooling. The secondary susceptibility reflects the changes in the fruit after cooling. Between them, these two classes cover all possible sources of susceptibility, and form a complete scheme of classification. Moreover, each class leads to a different type of "mortality curve". From primary susceptibility it is logical to expect the development of maximum injury without further increase

with time ; while from secondary susceptibility a gradual increase of injury corresponding to gradual changes in the stored fruit is expected.* In this knowledge a satisfactory means of analysing cold injury curves is provided.

It is not intended to review the literature of cold injury curves in the light of this simple scheme of analysis. A single example will suffice to illustrate the amount of information which may be gained, for which purpose the data obtained by Kidd and West (12, 13) on cold injury of apples will be used, since they are comparatively extensive :—

At all storage temperatures, from $-1\cdot0°$ C. upwards, cold injury appeared after a long interval of time and increased steadily without any approach to a maximum. Such development of injury is typical of secondary susceptibility, and it is inferred that the transition temperatures at the time of storage were below the lowest storage temperature, i.e. below $-1.0°$ C. Further, it was found that fruit which was cooled while passing through its respiratory climacteric was more susceptible than fruit in a pre- or post-climacteric phase. There can be alternative explanations of this result. The one explanation is that fruit which is cooled at the climacteric is more prone to those weakening changes in store which cause the transition temperature to rise—i.e. that such fruit bridges the gap between the initial transition temperatures and the storage temperature at a faster rate. Alternatively, one may assume that fruit at the climacteric has a greater primary susceptibility ; i.e. that the primary transition temperatures, although below $-1\cdot0°$ C., are higher than at the pre- or post-climacteric phase. In this case there would be a smaller gap to bridge by weakening changes between the initial transition temperatures and the storage temperature. The former alternative is the more probable. It was found that the difference between fruit cooled at the climacteric and other fruit was greater after forty-five weeks' storage than after thirty-nine weeks. This indicates that with fruit cooled at the climacteric there is that faster rise in transition temperatures which is required by the first alternative but not by the second. The weakness of fruit cooled at the climacteric thus seems to lie in the faster rate of change *after* cooling : the climacteric seems to affect secondary susceptibility, and there is, in these data, no direct evidence of any detrimental effect on primary susceptibility. It is possible that this faster rate of weakening is some relic of the fast rate of metabolic change which is normally associated with the climacteric at high temperatures. From this arises the question whether the rate of CO_2 production after cooling to very low temperatures is greatest for fruits that are cooled at the climacteric.

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* Under certain circumstances a maximum might conceivably be reached even with secondary susceptibility. For example, the process of ageing might in some cases result in a decrease of susceptibility, i.e. in a lowering of transition temperatures. This would stop the upward drift of the curve. At this stage, however, it does not seem profitable to discuss such eventualities until they are actually found by experiment.

SUMMARY.

Part I.

After relatively short periods in cold store, breakdown of plums, woolliness of peaches and pitting of Marsh grapefruit usually occur to a maximum extent at intermediate temperatures. At higher or lower temperatures there is less injury. That is, the injury temperature curves usually show a peak.

This peak seems to be a necessary consequence of the interaction of two opposing factors in the development of cold injury:—

(1) *An equilibrium factor.* Lowering the temperature increases the disposition towards injury.

(2) *A kinetic factor.* The higher the temperature the sooner does the injury become manifest, indicating a process governed by the thermo-chemical rule that the rate of change is reduced as temperature is lowered.

The temperature of maximum injury is labile and is not a fixed characteristic of the fruit. It may occur above or below the freezing point of the fruit. The latter cannot be demonstrated but must be assumed. The gradation from a peaked curve to an incomplete curve without demonstrable peak has been shown for Marsh grapefruit.

The three factors which lower the temperature of maximum injury are:—

(a) greater resistance of the fruit ;

(b) longer period of storage ;

(c) a faster rate of manifestation of injury.

Part II.

The number of fruits injured does not increase indefinitely as the period of storage is lengthened. A " maximum " is reached which is sufficiently definite to be considered as a first approximation.

The number of fruits injured when the maximum is reached is greatest at the lowest temperature, but the maximum is reached soonest at the highest temperature. Q_{10} values are given for the rate of development of injury.

The fact that a maximum is reached at each storage temperature indicates that the susceptibility to injury exists in the fruit at the time of cooling. This is referred to as " primary susceptibility ".

Frequency distribution curves of the " primary susceptibility " of a population of fruit over a range of storage temperatures can be prepared.

It is shown that the efficacy of any measure which reduces primary susceptibility may be measured in terms of shifting the frequency distribution curves of susceptibility down the temperature axis.

PART III.

The maximum injury referred to in Part II is not always a true maximum. Fruit changes during storage, and these changes result in further development of susceptibility to injury. This is referred to as "secondary susceptibility".

Injury due to primary and secondary susceptibility can be allocated. Various fruits may show only primary or secondary or both together.

The factors which predispose fruit to secondary susceptibility do not appear to be directly related to those causing primary susceptibility.

The simple scheme of analysis proposed is applied to the data of Kidd and West on cold injury of apples to illustrate the amount of information which may be gained.

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A NOTE ON THE USE OF α-NAPHTHALENE ACETIC ACID FOR ROOTING SOFT-WOOD CUTTINGS OF FRUIT TREE STOCKS

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CONSIDERABLE evidence has now accumulated from various parts of the world, including this country (Tincker (1)), to show that the synthetic growth substances introduced by the workers at the Boyce Thompson Institute, Hitchcock and Zimmerman (2), Van der Lek and Krijthe (3), are of considerable value in propagating plants from cuttings. Two main methods are commonly used for applying growth substances. Either a lanolin paste containing the substance is smeared on the cuttings or they are immersed in an aqueous solution for a short time before planting. Hitchcock and Zimmerman (2) have shown the second method to be much the more effective. In the present tests only this method was used. Experiments with cuttings of *Lonicera nitida* and *Prunus Cerasifera* var., Myrobolan B, using indoleacetic, indolebutyric and naphthalene acetic acids, indicated that α-naphthalene acetic acid is the most active compound of the three for promoting root formation. Hitchcock and Zimmerman (4), in a recent publication, have also shown that this substance is more effective than other synthetic growth substances for rooting cuttings, and in addition they state that salts of the acid are equally effective and less likely to prove toxic (to plant material) at high concentrations.

Work has therefore been begun at East Malling to determine whether the growth substances are also effective for fruit tree cuttings. Before starting large scale experiments in the open or in unheated frames, preliminary tests are being carried out in propagating frames with bottom heat. Here a considerable range of concentrations and times of treatment are being tried on soft-wood cuttings of a number of varieties of fruit tree rootstocks. Several different growth substances are being used. The object is to determine the best range of concentrations and periods of treatment, the most promising substances and suitable material to use in large scale experiments.

This note describes some of the results so far obtained with α-naphthalene acetic acid.

This acid is not readily soluble in water, so the solutions were prepared by dissolving it in 1-2 cc. of 95% alcohol, and making it up to the required volume with tap water. The treatment of the cuttings was simple. They were freshly made, their lower leaves being removed as it was found that these tend to rot if they come into contact with the solution. The basal inch of the cuttings was immersed in the solutions which were freshly prepared for each experiment. When testing a given species of cutting, solutions containing from 10 p.p.m. to 50 p.p.m. were generally used. After the period of immersion, which was usually from 6-18 hours, the bases were carefully rinsed in tap water, and the cuttings planted in Bedfordshire sand in a frame with bottom heat.

PRUNUS CERASIFERA SELECTION : MYROBOLAN B (PLUM ROOTSTOCK).

This variety is a fairly ready-rooting subject from hardwood cuttings. The trees from which the cuttings were taken had been potted up in the winter and placed in a heated greenhouse. The tips of terminal shoots in active growth were used as cuttings on April 24th and subjected in groups of five to various treatments. Four weeks later they were examined for rooting and it was found that a solution containing 30 p.p.m. α-naphthalene acetic acid with immersion of the bases for twelve hours had been most effective, all five cuttings being well rooted. Only one of the controls, all of which had been treated for a similar time with water, had one small root. On the treated series the roots arose not only from the end of the cutting but also from the stem above. (See Fig. 1.)

PYRUS COMMUNIS "FREE" PEAR ROOTSTOCK SELECTIONS.

(a) MALLING C.8.

From indoor material. Actively growing shoots were taken from potted material growing in a heated greenhouse, and were treated with various concentrations of α-naphthalene acetic acid in batches of five on April 24th. A solution containing 40 p.p.m. with immersion for twelve hours was very effective, all five cuttings rooting after five weeks, whilst in the untreated batch none had rooted (Fig. 2).

From outdoor layer beds. In view of the promising results obtained with pear cuttings taken from specially prepared material, cuttings taken from material growing in the open were tried.

Lateral shoots on which the terminal bud had just stopped growth were taken from current season's growth on layer beds on June 17th and treated with various concentrations of α-naphthalene acetic acid. There were twenty cuttings in each treatment. A solution containing 40 p.p.m. α-naphthalene acetic acid with immersion for twelve hours gave the best result. Fifteen of the cuttings were well rooted after four weeks, whilst the remaining five were just starting to root. The untreated control cuttings had callussed but none had rooted.

(b) MALLING C.2. (*From a tree growing in the open.*)

Lateral shoots which had just stopped growing were taken from a twelve year old tree on May 26th, and subjected to various treatments in batches of ten. Treatment with a solution of *a*-naphthalene acetic acid containing 30 p.p.m. and an immersion time of twelve hours caused five out of ten cuttings to root after five weeks, whilst none of the untreated controls had rooted.

OTHER FRUIT TREE CUTTINGS.

Other species were also similarly treated: With black currants (*Ribes nigrum*), which normally root readily from both hard and soft-wood cuttings, it was found that there was a marked acceleration in the time of rooting. Fig (*Ficus Carica*) cuttings, taken from a tree growing in the open, had rooted after two weeks, whilst the controls callussed but formed no roots during the same period.

The preliminary results described in this note show that treatment with *a*-naphthalene acetic acid, which proved the most effective of three growth substances tested, greatly stimulated rooting of certain fruit tree cuttings. The optimum strength of the solution ranged from 30-40 p.p.m., according to the species, when the time of immersion was about twelve hours.

The rooting of soft-wood cuttings of the Malling pear stocks, taken from material growing outdoors, is a very promising result, as these are acknowledged to be very difficult material from which to propagate vegetatively (5). These results were obtained with small numbers of cuttings and the use of bottom heat. It is intended to carry out further tests with larger numbers of cuttings to determine whether rooting can be induced and accelerated after suitable treatment when they are planted in the first instance in a cool frame in the open.

SUMMARY.

Preliminary tests on small numbers of soft-wood cuttings of certain plum and pear rootstocks and of black currant and fig indicate that immersion of the bases for twelve hours in 30-40 p.p.m. *a*-naphthalene acetic acid improved rooting markedly.

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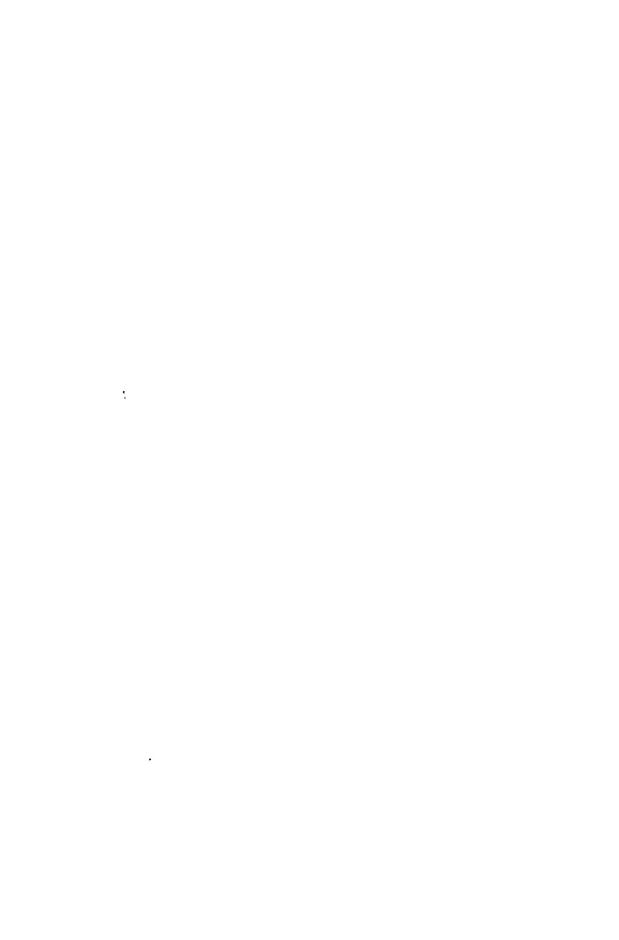
(1) *Tincker, M. A. H.* Experiments with Growth Substances, or Hormones, and the Rooting of Cuttings. Journ. Roy. Hort. Soc., 61, 510. 1936.

(2) *Hitchcock, A. E.* and *Zimmerman, P. W.* Effect of Growth Substances on the Rooting Response of Cuttings. Contrib. Boyce Thompson Inst. 8, 63. 1936.

FIG. 1

Rooting of soft-wood cuttings of Myrobolan B plum rootstock after four weeks. Upper row, untreated. Lower row, treated with a solution of a-naphthalene acetic acid, 30 p p m , for 12 hours

FIG. 2.

(3) *Van der Lek, H. A. A.* and *Krijthe, E.* Bevordering van de wortelvorming van stekken door middel van groeistoffen, with a summary: Stimulation of cuttings by growth substances. Mededeelingen van de Landbouwhoogeschool, Wageningen, 41. 1937.

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THE JONES-BATEMAN CUP FOR RESEARCH IN FRUIT CULTURE

IN 1920 Miss L. Jones-Bateman, of Cae Glas, Abergele, presented to the Royal Horticultural Society a valuable silver-gilt replica of the Warwick Vase to be used for the encouragement of fruit production. It was accordingly decided to offer it triennially for original research in Fruit Culture which has added to our knowledge of cultivation, genetics, or other relative matters, and it is available for award in 1937.

Candidates should submit accounts of their work by October 30th. The work dealt with should have been mainly carried out by the candidate in the United Kingdom, and mostly during the preceding five years. The cup will be held for three years by the successful candidate, who must give a bond for its safe return. The holder will be eligible to compete on the next or any succeeding occasion. When the cup is relinquished the holder will receive a Hogg Medal specially struck in gold.

The Assessors will be three, two appointed by the Royal Horticultural Society and one by the National Farmers' Union, who will report to the Council of the Royal Horticultural Society upon the originality and comparative potential value to the fruit-growing industry of the work of the candidates.

The Council of the Royal Horticultural Society will award or withhold the cup at its discretion.

A LABORATORY METHOD FOR TESTING THE TOXICITY OF PROTECTIVE FUNGICIDES

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INTRODUCTION.

FOR many years Bordeaux mixture and lime-sulphur have been the two standard protective fungicides, representing respectively two large groups, the copper- and the sulphur-containing fungicides. Each has, however, certain disadvantages as a spray for apple and pear trees. With Bordeaux mixture there is always a risk of russeting the fruit and scorching the leaves, even at dilutions containing as little as 0·05% copper. Lime-sulphur, on the other hand, frequently causes the dropping of fruit and leaves, especially when used post-blossom at 1% in hot weather. This tendency to cause damage has become increased in recent years by methods of spraying in which the fluids are delivered as driving sprays under high pressure.

There is thus a need either for the development of new fungicides or for the improvement of the old ones. Such improvement might be brought about by preparing the toxic element in some other form. Alternative preparations of copper and sulphur are already available as so-called " colloidal copper " and " colloidal sulphur ", and organic compounds represent a wide field for further development provided they can ultimately be economically used.

Past experience has shown that field testing of protective fungicides for fruit trees can be long and costly. The most important fungus disease of apples is Scab (*Venturia inaequalis* (Cooke) Wint.). It develops slowly, infection becoming severe only when climatic conditions favour it, and a whole year must elapse to complete a field test of any one preparation. In dry seasons, there may be insufficient infection to provide a reliable test, so that much of the work yields inconclusive results and has therefore to be repeated. The work described in the present paper was undertaken to ascertain whether a laboratory method could be devised that would enable time and money to be saved and at least permit reliable comparisons to be made as to the probable relative toxicities of old and of new protective fungicides.

It is not claimed that, by the use of the method proposed here, field trials are rendered unnecessary, but that much can be saved by eliminating unsatisfactory preparations at an early stage. Moreover, some idea can be obtained as to the minimum dilution toxic to the spores of the fungus. It is obvious that a method in which glass slides are used is limited in scope and may not be fully

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satisfactory because reactions between fungicide and leaf, or between fungus and leaf, are not involved.   Such reactions, however, will be encountered when promising materials are carried beyond preliminary laboratory tests to field trials.

LITERATURE.

For testing protectants many workers have used methods in which the fungus was brought into contact with the fungicide in suspension or in solution (Burrill (1), Hamilton (5), Liming (6), Uppal (15)), or in an agar culture-medium (Shapovalov (*), Palmiter (13)).   Although these methods give a certain amount of useful information, they do not give any indication of what may happen when the fungicides are sprayed on to a plant and allowed to dry. Doran (3) has shown that when conidia of *Venturia inaequalis* on the surface of an apple are sprayed with lime-sulphur solution, they are killed at a much lower concentration than when put on the dry deposit.   Moreover, with many protective fungicides, great changes take place on drying and exposure to air. The only methods suitable for comparing protectants, therefore, are those that make use of the dry deposit.

Burrill (1), in 1907, carried out germination tests on two sprayed cover-glasses, but the first to evolve a method on this basis were Reddick and Wallace (14) in 1910.   It consisted of spraying slides or cover-glasses with a material of known formula, drying and exposing them to the air, and putting spores in a drop of meteoric water on the dry deposit.   The method used in 1911 by Wallace, Blodgett and Hesler (16) was essentially the same, but special precautions were taken to ensure that the spraying was as uniform as possible.   Attention was paid also to standardizing the spore-concentration, and controls were retained on the unsprayed half of each slide.   The spores used were from Scab-lesions on apple leaves or fruit.   Doran (3), in 1922, used a similar method, incubating at 14° C., a temperature that he found most favourable for the germination of conidia of *V. inaequalis*.   This was followed in 1928 by the combined work of Butler and Doran (2) who used this method and obtained results on the toxicity to *V. inaequalis* of a number of arsenical preparations used alone and in combination with lime-sulphur.   These authors refer to much useful literature on the subject.   This method was further developed by McCallan (9) in 1930.   He took special precautions to distribute the spray evenly over the surface of the slides by using a hand-atomizer that delivered a fine, uniform, mist-like spray, and he claimed that, provided a certain minimum amount was applied, increase of it did not appreciably increase fungicidal efficiency.   The spore-suspension was applied as a drop from a pipette that delivered 20 drops per c.c.   Only one fungicide was placed in each moist

* The work of M. Shapovalov is unpublished, but is described by Morse (12, p. 178).

chamber. Martin (8), in 1932, surveyed the problems connected with both direct and protective fungicides, and, concerning the latter, he stressed the importance of temperature, oxygen relationships and the biological state of the spores. He issued the warning that " laboratory trials are dangerous tools and their value depends on what attention is paid to the tacit assumptions which they involve ".

A rather different method, with a limited application, was evolved by Goodwin, Salmon and Ware (4) in 1929, in which a water-suspension of zoospores of the Hop Downy Mildew (*Pseudoperonospora Humuli*) was placed on a dry spray-deposit and observed under the microscope. If the zoospores burst in a short time a good fungicide was indicated. This method seems suitable for use only with fungi that produce zoospores, and is therefore useless for the large majority of parasitic fungi. It is unsafe to conclude that a fungicide that is effective against one fungus will also be effective against others, hence each fungicide should be tested against the specific fungus it is required to combat.

GENERAL PRINCIPLES INVOLVED.

The method described below was developed for testing fungicides to control Apple Scab, and involved the use of conidia of the Fusicladium stage of the fungus *Venturia inaequalis* (Cooke) Wint. It was felt that the methods of other workers were not quite suitable for this purpose, mainly because the amount of spray applied was not sufficiently controlled. Any method that depends on visual estimation of uniform application must be open to criticism, especially when spray-substances having different wetting and spreading properties are used. Accordingly, a method of application was sought that would ensure precision in the amount of spray-material used in each experiment. This was obtained by applying a standard volume of diluted fungicide to a standard area of a standard surface. Glass was chosen as the most suitable surface because it is transparent and also enabled the method to be used throughout the year. With the possibility in mind of a reaction in the field between fungicide and leaf, which might influence fungicidal value, Marsh (7) used living leaves as well as cellulose-coated glass slides, and applied the spray-fluids with a precision spraying apparatus. He found that the living leaf " proved less flattering to the fungicide " than did the slide.

From the start (10), the guiding principles underlying the development of the method described below were: (a) standardization as far as possible of every phase in the process, to facilitate accurate repetition when desirable and to give uniform conditions for every preparation tested ; (b) simulation, as far as possible within the terms of (a), of the conditions encountered by fungus and fungicide in the field.

THE METHOD.

With a fine-bore glass pipette, 0·015 c.c. of the fungicide at the required dilution is applied to each of a number of circular areas 15 mm. in diameter, marked on a 3″ × 1″ glass slide. The fungicide is carefully spread to the edge of each area with a glass needle so that the area is completely covered by a film of liquid. This film is allowed to dry at room temperature, and is stored overnight at 20° C. A suspension in sterile distilled water of conidia from a pure culture is adjusted to a suitable concentration and is then dropped from a pipette at the rate of 0·04 c.c. on each area bearing the dry deposit of fungicide, and also on a number of clean areas as controls. The suspension is spread with a glass needle to cover each area completely, and the slides are placed in moist chambers, at 20° C., for at least twenty-four hours. The amount of germination is then recorded. When desired, washing with water to simulate the action of rain, is carried out after the fungicide has dried and before the suspension of conidia is applied.

Certain features of this method have required special study and are worthy of more detailed description. This will be found in the following paragraphs.

GLASSWARE.

Thin glass microscope slides of good quality were used. A circle of 15 mm. diameter was described with a writing diamond in the middle of each slide by rotating it in a lathe. Later, to save space and time, three such circles were drawn on each slide. Such slides can be obtained ready prepared, if desired, from commercial sources. It was found that 0·015 c.c. of liquid will cover each circular area and form an unbroken film on drying, except with liquids or suspensions of very high surface tension. With these, a small amount of some suitable " wetter " can be added to lower the surface tension, but in these experiments a different form of slide was used, the circular areas having a matt surface, produced by sand-blasting, thus reducing the surface tension between the liquid and the glass. The rough surface, however, is a disadvantage in that it renders it difficult to see whether or not the conidia have germinated. Staining with Gentian Violet or Cotton Blue makes matters easier.

The special pipettes used were made from glass tubes of fine bore, each fitted with a small rubber teat (such as are found in the B.D.H. Capillator Case for determining pH) ; they were graduated to deliver the required amounts.

The moist chambers were large, shallow Petri dishes, 6 in. in diameter, in which was placed sufficient distilled water to cover the bottom of the dish. Racks to support the slides were made by bending glass rod to the required shape.

All the glassware used was very carefully cleaned, especially the slides, to ensure that no foreign matter was present. Minute traces of grease are sufficient to prevent the fungicides from spreading over the prescribed area. Cleaning

involved the use of the following chemicals in order—warm dilute nitric acid (to remove fungicide-deposit) ; hot, 2·5% caustic soda (to remove grease) ; chromic acid cleaning solution (to remove organic matter) ; and ether (to remove any fats remaining). The slides were washed with water between each two operations, and were dried before being put into ether. Finally, they were transferred from ether to absolute alcohol, where they remained until required. Each was flamed and allowed to cool before the spray-fluid was applied. After tests in which organic fungicides had been used, the slides were washed in acetone, as this removes most of these materials.

Application of Fungicide.

With many fungicides, satisfactory cover of the test area can be obtained with dilutions in distilled water, but it was found that the suspension obtained in water with certain insoluble organic compounds tended to " cream ", making uniform distribution of the fungicide almost impossible. This was overcome by dissolving the pure compound in up to 2% acetone (usually 1%) and then emulsifying in a hand-operated machine with a 0·1% solution of " goulac " (sulphite lye). Goulac had been tested and found, up to at least 4%, to have no fungicidal effect on the conidia.

Washing the Spray-Deposit.

The earlier results, obtained with lime-sulphur and Bordeaux mixture, showed that, while the toxicity of various concentrations of the former approximated to that found in the field, that of Bordeaux mixture did not. Thus, a decrease in the concentration of lime-sulphur from 2·0% to 0·5% resulted in a great increase in the percentage of germinated conidia, while Bordeaux mixture did not allow free germination even when used as dilute as to contain only 0·025% copper sulphate (hydrated lime being present at the rate of three parts to two parts copper sulphate), which is a much lower concentration than that normally employed in the field. It seemed probable, therefore, that only a minute trace of a copper salt was required for toxicity, yet under field conditions, any considerable reduction in the copper sulphate content of Bordeaux mixture has resulted in decrease in the efficiency of this spray against Apple Scab. Under field conditions, a number of agents tend to reduce the amount of the deposit, the chief being rain. The rate at which rain water removes the deposit will influence the period during which the spray will remain effective. An attempt was made, therefore, by modifying the laboratory method, to obtain some indication of the duration of this period. For this purpose, a standard method of washing the deposit, after twenty-four hours' drying, was introduced.

An early method was to suspend the slides vertically from glass rods so that they were completely immersed in a large glass bath of distilled water. This method was preferred to that of washing with running distilled water chiefly for economy's sake, although the latter method would probably simulate more closely the action of rain. In the first experiment the distilled water was saturated with CO_2, but this proved so drastic that it entirely removed the deposit in a relatively short time. In subsequent experiments, therefore, fresh distilled water was used. Slides were removed at various intervals and allowed to dry. When a whole series was completed, all the slides were stored overnight in an incubator (20° C.), and next morning a spore-suspension was applied.

Later, it was found more satisfactory to submerge the slides in the water on horizontal glass racks with the surfaces bearing the spray-deposit facing downwards. The type of glass rack ultimately used could be gently rocked, and this prevented localized concentration in the water of any substance that might come from the deposit. This rocking also simulates to some degree the action of water flowing over the surface of a sprayed leaf. A rocking apparatus involving a pendulum, actuated by a water-jet, was set up and adjusted to give a standard number of swings of known amplitude per minute. This standard was found to vary somewhat with fluctuations in water-pressure and with the loading of the racks, hence an electric induction motor, driving a cam, was substituted as the power-unit as it could be run at constant speed irrespective of loading. The apparatus is illustrated in Fig. 1.

By this washing method, it was found that a Bordeaux mixture containing 0·025% copper sulphate remained toxic after two hours' washing, while one containing only 0·0125% copper sulphate was not effective after one hour's washing.

Lime-sulphur at 1% remained toxic after two hours' washing, but at 0·2% it was not effective, even unwashed. Reference to the results in Table IV to be found on page 263 shows that lime-sulphur at 0·33% (i.e. approximately 0·067% by weight polysulphide sulphur) was the lowest concentration effective, unwashed.

The figures obtained from these experiments are set out in Tables I and II.

The experiment with 0·0125% Bordeaux mixture was repeated on two other occasions and gave a similar result.

Very similar results had been obtained in the earlier experiments while the method was being developed.

All potential protective fungicides should be subjected to this washing test after the toxic limit of the unwashed deposit has been determined. If the concentration representing this toxic limit has to be greatly increased for the deposit to be effective after two hours' washing, the fungicide probably will not long remain effective under field conditions.

TABLE I.

Results of washing Bordeaux mixture deposits.

Fungicide and Washing Period.	Germination of Conidia.			Category.*
	Germ.	Inhib.	Ungerm.	
No fungicide (control)	100	0	9	5
0·025% Bordeaux mixture (0·006% Cu) Washed 2 hours ..	No germination.			1
Washed 1 hour ..	,, ,,			1
Washed ½ hour ..	,, ,,			1
Unwashed ..	,, ,,			1
0·0125% Bordeaux mixture (0·003% Cu). Washed 2 hours ..	41	32	70	2-3
Washed 1 hour ..	8	18	95	1-2
Washed ½ hour ..	No germination.			1
Unwashed ..	,, ,,			1

* See under " Methods of Assessing Results " on page 261.

TABLE II.

Results of washing lime-sulphur deposits.

Fungicide and Washing Period.	Germination of Conidia.	Category.*
No fungicide (control)	Fully germinated.	5
0·04% lime-sulphur. Washed 2 hours	,, ,,	5
Washed 1 hour	,, ,,	5
Unwashed	,, ,,	5
0·2% lime-sulphur. Washed 2 hours	,, ,,	5
Washed 1 hour	,, ,,	5
Unwashed	Mainly germinated with short germ-tubes.	4
1·0% lime-sulphur. Washed 2 hours	No germination.	1
Washed 1 hour	,, ,,	1
Unwashed	,, ,,	1

* See under " Methods of Assessing Results " on page 261.

STANDARDIZATION OF FUNGUS.

As it is not easy to obtain natural supplies of conidia of *V. inaequalis* all the year round, and as naturally occurring conidia tend to be very variable in germination, they must be obtained from artificial cultures. In the present work the cultures were started from single conidia obtained from Scab-spots on the leaves of Bramley's Seedling, and a fresh isolation was made each season. This fungus does not readily produce conidia on many media, and its growth-rate is slow even on a favourable medium. An agar plate medium proved unsuitable owing to the difficulty of removing the conidia from the surface of the medium with sterile water ; hence it was discarded. Agar films were produced by horizontally rotating test-tubes each containing a small quantity of warm agar. Although these yielded a satisfactory suspension of conidia in sterile water, their use was ultimately given up because they were difficult to handle, and pieces of the film became detached. Pieces of sterilized apple wood in test-tubes were found to be the most suitable medium, as conidia were produced plentifully and were easily detached by water. These pieces were obtained by splitting three-inch lengths of three- or four-year-old apple branches from which the bark had been peeled, and they were then placed singly in test-tubes with a little water and autoclaved.

At first the pieces of wood were inoculated by spraying from an atomizer with a suspension of conidia in distilled water, but later it was found more satisfactory simply to pour into the tube a quantity of the suspension, which was then shaken up so that the surface of the wood was uniformly wetted. Trials were made with suspensions of conidia in nutrient solutions instead of in distilled water. The solutions tested contained 10% dextrose, 10% laevulose, 10% sucrose, 5% sucrose, 1% malt extract and 0·5% asparagin. Although slight differences were recorded in the numbers of conidia produced and in their capacity for germination, they were too small to be significant.

The pieces of wood must be kept almost saturated with water, for if they become too dry the fungus grows very slowly, and if too wet, the fungus produces a mass of mycelium and few conidia. The optimum moisture conditions can be found only by experience, but if the wood is saturated with water before autoclaving and about 1 c.c. of conidia-suspension is introduced when inoculating, the resulting culture should give sufficient conidia in fourteen days. If the cultures are shaken daily, conidia are produced more plentifully than if the tubes are not disturbed.

Before experience of this method had gone very far, considerable variations in germination capacity and germ-tube vigour were found among different conidia-suspensions. At first this was thought to be connected with the age of the conidia and cultures of various ages were tested. It was then found that while cultures fourteen days old produced large numbers of conidia that

germinated well, very old cultures gave a high proportion of spores that did not germinate. All the cultures subsequently used, therefore, were about fourteen days old. The percentage of conidia that germinated, however, still varied very much between one culture and another, and eventually it was found that nutriment was extracted from the pieces of wood while the suspension was being made, and that the amount extracted varied with each occasion. In order to counteract this, the conidia were washed. The suspensions were centrifuged until the conidia collected at the bottom of the tube, after which the water was poured off and the tube was refilled with distilled water. This process was repeated three times, and the suspension was finally adjusted to contain approximately 50 conidia per field of 2 mm. diameter. A suspension prepared in this way gave excellent germination and very consistent results. Recently, however, a series of these suspensions showed no germination, and the cause was finally traced to the distilled water, which had been obtained from a silver still. A minute trace of some toxic substance must have been introduced from this still, for when the water was re-distilled from a glass vessel, ready germination was restored. Conidia, when suspended in distilled water with a trace of nutriment, appear to be able to withstand the toxic effects of the fungicide-deposit better than when they are in pure distilled water.

APPLICATION OF CONIDIA-SUSPENSION.

In the method as described in a previous paper (10), 0·05 c.c. of the conidia-suspension was put on each area. Later, it was found that this amount sometimes overflowed the delimited area and this undesirable effect was successfully overcome by reducing the volume to 0·04 c.c.

METHODS OF ASSESSING RESULTS.

It was necessary to examine the conidia with a $\frac{1}{6}''$ objective and ×6 ocular because the density of the deposit at the higher concentrations made it very difficult to see them with a low power. Ten fields or traversed portions, each containing approximately ten conidia, were taken at random on each circular area and the germination was recorded. Three categories of conidia were counted, (a) those ungerminated, (b) those with a germ-tube not longer than the conidium (inhibited* after initial germination), (c) those with a germ-tube longer than the conidium. The first examination was generally made after twenty-four hours' incubation, but some of the replicates were always left in the incubator for at least forty-eight hours to ensure as complete germination as possible.

When a large number of substances have to be dealt with, it is desirable to have a more rapid method than that of counting individual conidia, especially

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* These conidia were not " late starters " for they were subsequently examined at intervals and no further growth was observed.

for preliminary sorting, when only wide differences are needed.   Hence germination was classified by inspection into categories as follows:—

1. All ungerminated or with very few inhibited.

2. A mixture of ungerminated and inhibited in roughly equal proportions.

3. Inhibited predominating, with some ungerminated and some germinated.

4. Germinated predominating, with a considerable number inhibited and a few ungerminated.

5. Practically all germinated.

Considerable experience of the counting method is needed before accuracy in estimating germination by this category method is achieved.   The counting method is essential also for the critical determination of the minimum toxic concentration of any fungicide.

RESULTS.

That the results of each experiment in comparable series are in close agreement is demonstrated by the category figures obtained from specific tests repeated several times.   These will be found in Table III.

TABLE III.

	Experiment No.	1.	2.	3.	4.	5.
Series I. No fungicide (control)		5	5	5	5	5
Colloidal copper preparation (6% Cu)	0·0003% Cu			4·5		
	0·0004% Cu			3		
	0·0006% Cu	2-3	1-2	1-2	1	3
	0·0018% Cu		1			
	0·003 % Cu		1		1	
	0·006 % Cu	1			1	1
	0·06 % Cu	1				
Series II. No fungicide (control)		5	4	5	5	
Copper sulphate	0·0003% Cu		1	1-2	1-2	
	0·0016% Cu	1				
	0·0025% Cu		1	1	1	
	0·0031% Cu	1				
	0·006 % Cu	1				
	0·025 % Cu		1	1	1	
Zinc sulphate	0·00022% Zn		3	5	5	
	0·0022 % Zn		2	3	4	
	0·022 % Zn		1	1	1	

Table IV contains a selection from the results so far obtained by the use of the method described, the deposit of fungicide in every case being unwashed.

The headings of the three columns in the Table are to be interpreted as follows:—

Spray-fluid not effective : Germination in categories 4 and 5, germinated conidia predominant.

Spray-fluid partially effective : Germination in categories 2 and 3, inhibited and ungerminated spores predominant, and only a few germinated conidia.

Spray-fluid effective : Germination up to but not including category 2, only a small proportion of inhibited and no germinated conidia being included.

TABLE IV.

Preparation.	Not effective. (Cat 4-5) at :	Partially effective. (Cat 2-3) at ·	Effective (Cat. 1) at ·
Containing copper ·			
Copper sulphate		0·0003% Cu	0·0016% Cu
Bordeaux mixture			0·003% Cu
Colloidal copper preparation (6% Cu)	0·00045% Cu	0·0006% Cu	0·0018% Cu
Containing zinc :			
Zinc sulphate	0·002% Zn		0·022% Zn
Zinc-lime	0·41% Zn		0·81% Zn
Zinc-lime-" goulac " preparation (6·7% Zn)		0·11% Zn	0·23% Zn
Containing sulphur :			
Lime-sulphur	0·04% S†		0·067% S†
A 40% sulphur-dispersion A	0·001% S	{ 0·01% S and { 0·1% S	
,, ,, ,, B	0·01% S	0·1% S	
,, ,, ,, C	0·001% S	{ 0·01% S and { 0·1% S	
Organic compounds :			
Para-acetoxyphenyl*iso*thiocyanate	0·001%	{ 0·01% and { 0·1%	
α-Chloro-β-naphthol	0·01%	0·1%	
8-Hydroxyquinoline			0·001%*
2-Mercaptobenzthiozole	0·001%	0·005%	0·01%
Methyl " dixanthogen "	0·1%		
β-naphthol	0·1%		
Salicylanilide	0·002%	0·003%	0·004%
Tetramethylthiuram sulphide	Inconclusive		0·005%
Tetramethylthiuram disulphide	0·0001%		0·0005%
Thiodiphenylamine	0·05%		0·1%

* Lowest concentration tested ; the other figures in this column are the lowest effective concentrations.

† S in this case is sulphur in polysulphide form.

DISCUSSION OF RESULTS.

The results obtained by this method suggest that it is reliable for comparing fungicides in the laboratory. Whether there is any correlation between them and those that would be obtained in the field is a subject for future investigation.

The following comparisons show that it is unlikely that a single numerical factor will be found by which the effective concentration of any fungicide determined in the laboratory can be multiplied to give the concentration necessary for effective use in the field. With lime-sulphur, the lowest effective concentration in the field (1%) is three times that of the lowest effective concentration in the laboratory (0·33%, unwashed). Similar comparison of Bordeaux mixture in the field (0·2% copper sulphate) and in the laboratory (0·0125% copper sulphate) gives a factor of sixteen. Even when the concentration (0·025% copper sulphate) effective after two hours' washing is used for the comparison, the factor is still as high as eight. It seems very probable, therefore, that with widely differing fungicides such as lime-sulphur and Bordeaux mixture, a different ratio between the results obtained in laboratory and field will be found for each.

With organic compounds, concentrations above 0·1% were not considered, because the high cost of these compounds renders their use prohibitive unless they are effective at low concentrations. The promising organic compounds among those tested, therefore, were 8-hydroxyquinoline, salicylanilide, tetramethylthiuram sulphide and tetramethylthiuram disulphide. In a field trial in 1935 (11), a spray containing 1% of a 15% dispersion of tetramethylthiuram sulphide, though more effective than one containing 1% of a 25% dispersion of salicylanilide, was not as effective as the standard lime-sulphur spray (2·5% pre-blossom ; 1% post-blossom) in controlling Apple Scab. Since, in laboratory trials, tetramethylthiuram disulphide proved more fungicidal than tetramethylthiuram sulphide, it seemed well worth a field trial. This compound has therefore been included in one in 1937, and 8-hydroxyquinoline will be tested as opportunity offers.

SUMMARY.

1. The literature relating to laboratory methods for testing protective fungicides is reviewed.

2. The following method has been devised and tested : With a fine-bore pipette, quantities of 0·015 c.c. each of the liquid fungicide are spread uniformly over circular areas, each 15 mm. in diameter, delimited on glass slides. When the liquid has evaporated and the deposit is dry, 0·04 c.c. of a suspension in water of washed conidia from a pure culture of *Venturia inaequalis* is applied in a similar manner, and the slides are then stored in a saturated atmosphere

at 20° C. At intervals counts of germination are made. When desired, the spray-deposit is washed with water before the conidia-suspension is applied.

3. A method of growing *Venturia inaequalis* in pure culture for the production of conidia is detailed, as well as one for freeing the conidia-suspension from nutriment by centrifuging.

4. A method is presented for rapidly assessing the results of treatments by classifying the mass appearance of conidia-germination into five categories.

5. The results of a number of tests with copper- and sulphur-containing inorganic substances, and also with certain organic compounds are tabulated and discussed. Amongst the last-named, tetramethylthiuram disulphide was found to be very highly fungicidal.

ACKNOWLEDGMENTS.

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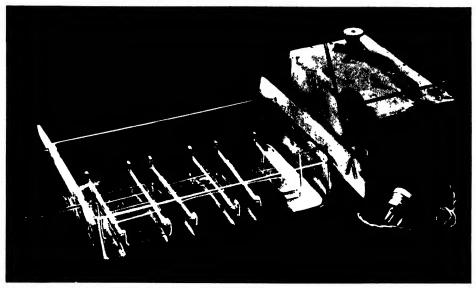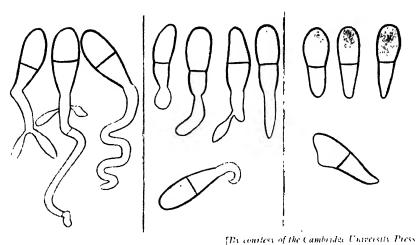
FIG. 1
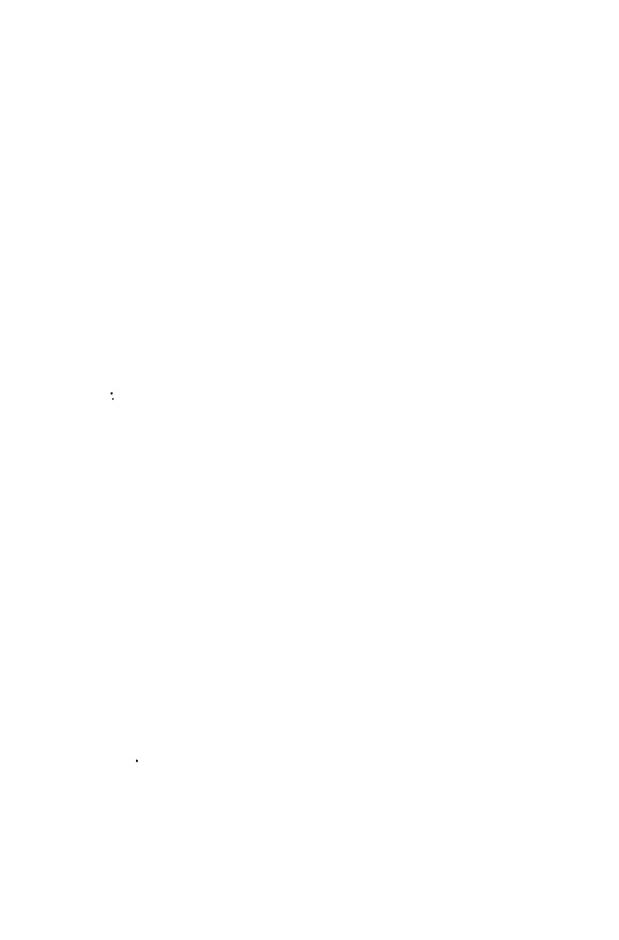
Apparatus for washing deposit of spray-fluid on glass slides. An electric induction motor is housed in the box and rotates the reel from which the drive is transmitted to the cam by a belt. The glass rod is held in the grooved edge of the cam-wheel and transmits the rocking motion to the racks

(approx ¼ actual size)

FIG. 2.

Illustrating conidia of *Venturia inaequalis* classed as germinated (left), inhibited (centre) and ungerminated (right).

STUDIES IN INCOMPATIBILITY BETWEEN STOCK AND SCION, WITH SPECIAL REFERENCE TO CERTAIN DECIDUOUS FRUIT TREES*

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I. INTRODUCTION.

The problem of incompatibility between stock and scion is one which has puzzled propagators and pomologists all over the world ever since grafting and budding began to be employed. Thus, in China, as early as about A.D. 500, Chia Su-lih (7) wrote in his book *Chi Ming Yao Suh* (Methods of Progressive Farming) that the pear, *Pyrus serotina* Rehd., is best suited on "Tu Li", *Pyrus phaeocarpa* Rehd., and that the bark of the scion must be brought into close contact with that of the stock in order to get good union. He also mentioned that the plum, *Prunus salicina* Lindl., can successfully be grafted on peach stocks, *Prunus persica* Lindl., and will bear prolifically, whereas the peach usually fails to grow on plum stocks, *Prunus salicina* Lindl. Accounts in old French and English literature also mention the phenomenon of incompatibility. Thus, Thomas Andrew Knight (32), writing in 1812 on the effect of different kinds of stocks in grafting, stated that plum stocks are best adapted to the peach, and apricot stocks to the Moorpark Apricot. He said that his Moorpark Apricot budded on apricot stock had smooth bark and a polished surface, and that the whole tree presented a greater degree of health and vigour than did trees budded on any other stock.

This problem of incompatibility is a serious handicap to the nurseryman in choosing suitable stocks. From the point of view of the practical grower, it is a disadvantage when big trees, on reaching bearing age, are suddenly broken off at the union in a storm. It is also fairly common to see 8- or 10-year-old trees gradually decline both in fruiting and in vegetative vigour so that finally they have to be grubbed.

Most fruit trees are budded or grafted, i.e. are made up by combining a scion with a stock. If there were any abnormal manifestations or obstructions at the union causing trouble to the vital growth-processes of the tree, then either the scion or the stock effect may be interfered with. Therefore, a closer

* The present paper is an abridgment of a Thesis (presented in August 1937) approved for the Degree of Doctor of Philosophy in the University of London.

study of the symptoms and possible causes of incompatibility may afford some explanation of certain stock-scion relations.

Survey of the literature. ⏐ Since the end of the nineteenth century many attempts have been made to explain the problems of incompatibility. Thus, Waugh (64), Proebsting (43, 44), Heppner and McCallum (23, 24) and Bradford and Sitton (4), attributed incompatibility chiefly to structural weakness and abnormal deposits at the union.⏐ Daniel (10), Gleisberg (12), Crafts (9), and Mendel (39), attributed it mainly to differences in the anatomy of stock and scion, ⏐ while Guignard (15), Rives (48), Green (14), Hofmann (25), Proebsting and Barger (45), Haas and Halma (16) and Toxopeus (61), believe that the cause is of biochemical origin. Hafekost (17), working with pears grafted on East Malling quince selections D, E, F, G, stated that there was a difference in the power of protoplasmic reaction between stock and scion, resulting in a higher or lower concentration of the cell sap. Kostoff (33, 34, 35), on the other hand, from his results with intergeneric grafts of Solanaceae, concluded that the toxins produced by different antibodies induced by the presence of antigens in different kinds of plants are responsible for incompatibility. None of these interpretations has been finally accepted, and up to the present there is still no method of predetermining the compatibility or otherwise of any given combination of stock and scion.

Botanical relationship not a reliable guide. Older conceptions regarded botanical relationship as a fair guide to the compatibility of the stock and scion. Baltet (3) held that plants belonging to the same botanical group may successfully be grafted. But plants of different species or even of different genera, such as lilac (*Syringa*) on a kind of privet (*Ligustrum lucidum* Lindl.), or Citrus on *Poncirus trifoliata* Lindl., are commonly and successfully worked in China. On the other hand, Hatton (19) found that different selections of quince stocks belonging to the same species (*Cydonia vulgaris* Lindl.), showed marked differences in regard to their suitability for pears. Later reports from East Malling (20, 21, 22) concerning pears, plums, and peaches, also showed clearly that some plum varieties (*Prunus domestica* L.) show a less degree of compatibility with Common Plum stock (*Prunus domestica* L.) than with a Cherry-Plum selection, Myrobolan B. (*Prunus cerasifera* L.). Very different results have been obtained even among selections bearing the same varietal name, such as St. Julien Plum. Thus, while the peach Hale's Early has grown satisfactorily on St. Julien A and St. Julien C, it has proved completely incompatible with St. Julien K. From this evidence it would appear that incompatibility cannot be directly correlated with any taxonomical relationships.

Disease symptoms and signs of incompatibility must be distinguished. Quite often a tree dies because of infection with some kind of disease-producing organism

at the union or in the rootstock portion of the tree. Again, it is not uncommon for a tree to die suddenly from excessive heat or from waterlogging. Riker and others (47) have clearly shown how overgrowths and other malformations resembling Crown Galls at the union in root-grafted apple trees may result as an after-effect of wounding. Similarly, Rawlins and Horne (46) have described how in cherries the wound made in grafting may act as a place of entry for disease, and Wormald (65) has proved that a wound is the primary place of entry for the Bacterial Die-Back organism in plum trees. Pitmaston Duchess pear has been looked upon as a compatible scion on Quince C, but in the summer of 1936, as many as 50 per cent. of such grafted trees died within a short period in the nursery at East Malling. On examining the dead trees, the bark of the scion was found to be badly blistered, and many cells inside the wood were blackened. The stock portion was healthy and white inside. It was found that two trees were badly cankered above the union.

Percentage of take not a reliable indication of incompatibility. Percentage of bud and graft take is sometimes very variable. Climatic and environmental conditions during the time of budding and grafting, the condition and size of the stock, the maturity of the scion wood, the date and method of propagation are all factors which influence the percentage of take. Work conducted at East Malling by Amos and others (1) has shown that the percentage of bud take cannot always be depended upon as a fair indication of incompatibility. For instance, Czar Plum budded on Common Plum showed about 37% take in 1933, 70% in 1934, and 0% in 1935. Similarly, Pitmaston Duchess pear budded on Quince F had 100% take in 1934 but only 62% in 1935.

Again, the percentage of bud take may vary considerably according to the date of budding and the method of handling the buds. To illustrate this point, buds of Hale's Early Peach were inserted into three different plum stocks at weekly intervals from June 24th to August 26th, 1936. Those on one of the stocks, Common Plum, showed striking variations in the percentage take according to the date of budding. Thus, the numbers of buds still alive on March 15th, 1937, of each ten, budded on three stocks on the dates mentioned, was as follows :

	24/6	1/7	8/7	15/7	22/7	29/7	5/8	12/8	19/8	26/8
Common Plum ..	0	0	1	3	4	1	3	3	6	8
Brompton ..	8	10	10	10	9	10	10	10	9	9
Myrobolan B ..	7	8	8	10	10	8	10	10	10	10

Eventually, all the trees on Common Plum died at the end of May, and, at the end of August 1937, those on Myrobolan B appeared likely to die.

Work in Chekiang, China, (6) has shown that the percentage of bud take of Bartlett and Kieffer pears on *Pyrus calleryana* stocks varies markedly with the

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humidity, rainfall and diurnal temperature during the period of budding. It is also known that climatic and environmental conditions influence the manifestations of incompatibility to a great extent, even after the buds have taken and the scions have started growth. Thus, while it is well known that the apple will not normally grow for more than a few seasons when grafted on quince stocks, a recent communication from H. A. Urbina, in Uruguay, states that two varieties, Winter Banana and Reineta de Verano, have been growing well on certain quince stocks for over twenty years, and are still producing about 120-150 kilograms of fruit per tree per annum. Mitchurin (40) states that a lemon tree has been successfully grafted on a pear stock and is still living and growing after six years.

There is some evidence that certain combinations will grow in one locality but may totally fail in another. In such cases it would appear that incompatibility exists, but may not always reveal itself morphologically owing to favourable growth conditions. Unless the condition at the union is closely examined, too hasty a judgment must not be made by looking at the performance of the top growth. It may therefore safely be assumed that the symptoms of incompatibility appear most quickly and in the severest manner when the tree is grown under the most adverse environmental conditions. On the other hand, under favourable conditions, the symptoms may take some time to appear, although incompatibility actually exists in the tree. Argles (2) suggested that "incompatibility may be regarded as an inherent antagonism or discordant association between certain stocks and scions, the cause or causes of failure or abnormalities arising out of the nature of two plants, and the form taken by the failure being governed in greater or less degree by the subsidiary factors of environment and treatment. Thus, under external conditions which favour union and growth, failure will tend to be delayed, while under unfavourable conditions of environment and treatment it will tend to be accelerated."

II. Symptoms of Incompatibility.

In order to study the various manifestations of incompatibility in growth and other internal abnormalities both in supposedly compatible and incompatible grafts and buds, some eighty-six graft combinations of pear, apple, quince, plum, peach, and cherry scion and stock varieties were made in the spring of 1936, and thirty-two bud combinations of pear, quince, plum, and peach scion and stock varieties were made in the summer of that year. The rootstocks were of clonal origin, propagated vegetatively at East Malling. The stock and scion varieties used and mentioned below were selected to represent two main groups, those generally considered compatible and those generally considered incompatible, as described by Amos and others (1):

Pears :

Compatible combinations :
    Conference on Quince A and C.
    Durondeau on Quince A and C.
    Pitmaston on Quince A and C.

Partially incompatible combinations :
    Conference on Quince F and G.

Incompatible combinations :
    Durondeau on Quince F and G.
    Pitmaston on Quince F and G.

Plums :

Compatible combinations :
    Victoria on Myrobolan B.
    Victoria on Common Plum.
    Czar on Myrobolan B.
    President on Myrobolan B.

Incompatible combinations :
    Czar on Common Plum.
    President on Common Plum.

Peaches :

Compatible combinations :
    Hale's Early on Brompton.
    Hale's Early on St. Julien A and C.

Incompatible combinations :
    Hale's Early on Myrobolan B.
    Hale's Early on St. Julien K.

Cherries :

Compatible combinations :
    Prunus serrulata on Mazzard.
    Prunus pseudocerasus on Mazzard.

Incompatible combinations :
    Prunus serrulata on Mahaleb.
    Prunus pseudocerasus on Mahaleb.

These combinations were made up in three different ways, viz. scion on stock, stock on scion, and stock on stock, in order to observe any symptoms of incompatibility in the different combinations and particularly to see whether the same symptoms appeared in the reciprocal combinations.

1. *Initial take and subsequent growth of grafts and buds.*

All the above mentioned stocks were of the same age in each comparable group, and were selected for uniformity in the field. The scions and buds were taken from the middle portions of shoots of uniform maturity from trees of the same age.

When grafting, the stocks were arranged in rows so that two replications could be made for every combination. The stocks of the budded trees had been planted in 1935 in randomized blocks, and four replications were made of each combination.

To ensure uniform manipulation all buds and grafts were inserted by the writer at the same height (approximately 6 cm. above ground level) and position (on the north side) on each stock by the same methods, viz. shield, and whip and tongue respectively.* All grafts or buds in each group were inserted on the same day and wax was applied to the grafts. All the after-treatment in the nursery was uniform throughout each group. The weather conditions during grafting and budding in 1936 were normal. The soil moisture in the nursery, as indicated by meter readings, showed that there was no lack of moisture in the soil through-out the growing season following grafting and budding.

(*a*) *Grafted trees.* At the end of the growing season (December 1936) the new wood growths of each combination were measured. The percentage take and the average growth of new wood for each combination are shown in Tables I-IV :

TABLE I.

Percentage take and Maiden Growths of Pear and Quince Grafts.

Combinations		No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
Conference on Quince A	..	20	19	95	134·8	*13·8*
Conference on Quince G	..	20	19	95	109·3	*8·9*
Durondeau on Quince A	..	20	19	95	100·4	*20·1*
Durondeau on Quince G	..	20	19	95	160·0	*17·6*
Quince A on Durondeau†	..	20	18	90	171·9	*84·4*
Quince G on Durondeau†	..	20	2	10	90·7	*36·4*
Quince A on Quince A	..	20	16	80	336·3	*92·6*
Quince A on Quince G	..	20	17	85	625·3	*88·8*
Quince G on Quince A	..	20	7	35	487·3	*120·8*
Quince G on Quince G	..	20	19	95	705·2	*106·7*

* In budding, any small portion of wood that might accompany the bud was not removed before insertion.

† These trees were already budded on Quince A.

TABLE II.

Percentage take and Maiden Growths of Plum and Plum Stock Grafts.

Combinations.		No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
Victoria on Myrobolan B	..	15	15	100	385·0	*53·1*
Victoria on Common Plum	..	15	14	93	117·5	*10·6*
Czar on Myrobolan B	..	15	14	93	556·1	*87·0*
Czar on Common Plum	..	15	7	47	61·7	*12·8*
Myrobolan B on Czar*	..	20	17	85	460·6	*53·3*
Common Plum on Czar*	..	20	8	40	81·8	*13·1*
Myrobolan B on Myrobolan B		15	15	100	2,137·1	*406·3*
Myrobolan B on Common Plum	.. .. ..	15	13	87	320·7	*40·8*
Common Plum on Myrobolan B		15	14	93	500·5	*45·2*
Common Plum on Common Plum	.. .. ..	15	14	93	138·3	*6·9*

* These trees were already budded on Myrobolan B.

TABLE III.

Percentage take and Maiden Growths of Hale's Early Peach and St. Julien Grafts.

Combinations.		No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
Hale's on St. Julien A	..	10	5	50	531·3	*69·1*
Hale's on St. Julien K	..	10	1	10	85·0	—
St. Julien A on Hale's*	..	20	20	100	656·7	*54·7*
St. Julien K on Hale's*	..	20	3	15	87·5	*22·5*
St. Julien A on St. Julien A		10	10	100	375·6	*45·9*
St. Julien A on St Julien K		10	4	40	321·7	*63·3*
St. Julien K on St. Julien A		10	8	80	321·4	*25·3*
St. Julien K on St. Julien K		10	9	90	458·3	*116·8*

* These trees were already budded on St. Julien A.

TABLE IV.

Percentage take and Maiden Growths of Cherry and Cherry Stock Grafts.

Combinations.		No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
Serrulata on Mazzard	..	20	20	100	166·7	*12·7*
Serrulata on Mahaleb	..	15	0	0	—	—
Pseudocerasus on Mazzard	..	20	20	100	170·7	*20·9*
Pseudocerasus on Mahaleb	..	15	4	27	107·7	*14·2*
Mahaleb on Mahaleb	..	15	15	100	1,982·6	*275·1*
Mahaleb on Mazzard	..	20	19	95	559·8	*51·5*
Mazzard on Mahaleb	..	15	15	100	270·9	*42·0*
Mazzard on Mazzard	..	20	20	100	131·6	*14·2*

The variety of *Prunus serrulata* used was Ukon; that of *Prunus pseudocerasus* was East Malling F269. The Mazzard used was F5/1, and the Mahaleb was F8/1/10 (both Malling selections).

(*b*) *Budded trees.* Budding was done in August 1936. All the living buds were counted twice after bud take, once in October 1936 and again in April 1937. The final percentages of bud take and first year's growth are presented in Tables V-VII.

TABLE V.

Percentage Bud take and Maiden Growth of Pears and Quinces.

Combinations.		No. budded.	No. take.	% take.	Wood growth.†
					cm.
Conference on Quince A	..	32	31	97	71·3 ⎫
Conference on Quince G	..	32	31	97	87·9 ⎬
Durondeau on Quince A	..	32	31	97	79·4 ⎫
Durondeau on Quince G	..	32	32	100	31·5 ⎭
Quince A on Durondeau*	..	30	21	70	—
Quince G on Durondeau*	..	30	18	60	—
Quince A on Quince A ..	..	32	32	100	844 ⎱
Quince A on Quince G ..	..	32	28	88	800 ⎰
Quince G on Quince A ..	..	32	27	84	485 ⎱
Quince G on Quince G ..	..	32	32	100	432 ⎰

* These trees were already budded on Quince A.

† Pairs showing insignificant differences in wood growth are bracketed together.

TABLE VI.

Percentage Bud take of Hale's Early Peach and Plum Stocks.

Combinations.			No. budded.	No. take.	% take.
Hale's Early on Brompton	..	..	24	23	96
Hale's Early on Myrobolan B	..	..	24	11	46
Brompton on Hale's Early*	..	..	30	26	87
Myrobolan B on Hale's Early*		..	30	21	70
Brompton on Brompton	..	..	24	24	100
Brompton on Myrobolan B	..	..	24	24	100
Myrobolan B on Brompton	..	..	24	24	100
Myrobolan B on Myrobolan B	..		24	24	100

* These trees were already budded on St. Julien A.

TABLE VII.

Percentage Bud take and Maiden Growth of Plums and Plum Stocks.

Combinations.	No. budded.	No. take.	% take.	Wood growth.*
				cm.
Victoria on Myrobolan B	48	44	92	194
Victoria on Common Plum	48	44	92	122
Czar on Myrobolan B	48	48	100	272
Czar on Common Plum	48	17	35	66
President on Myrobolan B	48	43	90	342
President on Common Plum	48	15	31	44
Myrobolan B on Myrobolan B	48	48	100	819
Myrobolan B on Common Plum	48	41	85	417
Common Plum on Myrobolan B	48	47	98	485
Common Plum on Common Plum	48	43	90	208

* In every case the trees on Myrobolan B have made significantly more growth than those on Common Plum.

From the data presented in Tables I-VII, it is clear that the same manifestations of compatibility and incompatibility were displayed in both stock scion and scion-stock combinations, and examples are illustrated in Figs. 1 and 2. This is contrary to the experience of Chandler (5) with cherries, Heppner and McCallum (24) with plums, and Toxopeus (61) with Citrus.

Among all the combinations tested, there was a somewhat lower percentage take on grafted than on budded trees. Moreover, the difference in percentage take between compatible and incompatible combinations seems much wider among grafted than budded trees, as is clearly shown between Quince A and Quince G on Durondeau and Hale's Early Peach on St. Julien A and St. Julien K. However, there was a clear indication of high percentage of bud failure on very highly incompatible combinations, such as Quince G on Durondeau. In other less acute cases, such as Durondeau on Quince G, budding seems to afford very much less indication of incompatibility, judging from the percentage of bud take ; nevertheless, decline in vigour invariably appeared at a later date.

There was a certain amount of variation in the time of appearance of incompatibility and in its degree of severity (or in both), between the scion-stock and the stock-scion combinations in each group. For instance, the symptoms showed up earlier and were more marked in Quince G on Durondeau than in Durondeau on Quince G. Similarly, Common Plum on Czar, showed incompatibility much earlier than Czar on Common Plum, Hale's Early Peach on Myrobolan B than Myrobolan B on Hale's Early Peach, and St. Julien K on Hale's Early Peach than Hale's Early Peach on St. Julien K.

The stock on stock series also showed some degree of incompatibility in those combinations in which the incompatible stock was used as the scion. Thus,

Quince G grafted on Quince A made much less new wood growth in the first year than Quince G on Quince G. On the other hand, the reciprocal grafts of Quince A on Quince G made more growth in the first year than when worked on themselves. In neither case, however, are these indications confirmed by the growth of the budded trees.

Finally, it is an interesting point that although Hale's Early Peach and Myrobolan B are reciprocally incompatible, it was observed that the buds of Myrobolan B on Hale's Early started to develop in the autumn after budding, and reached a length of about 3 cm. Myrobolan B budded on Myrobolan B and on Brompton behaved in the same way. But the buds of Hale's Early Peach on Myrobolan B started to grow at a much later date in the spring of 1937 than did those on Brompton. The same phenomenon was observed on Czar and President plums budded on Myrobolan B and Common Plum respectively. This probably indicates that there is a lack of affinity at the union in incompatible combinations, even in the dormant bud stage just after budding. It is also conceivable that the Myrobolan B, an early and active grower, has retained its potentially early growth somewhat independently of the double worked stock, i.e. of Hale's Early on St. Julien A.

2. *Premature autumn coloration of the leaves, premature flower bud formation, early defoliation and dying back of the young growths.*

All the incompatible combinations in these series showed the general symptoms of premature autumn coloration of the leaves, premature flower bud formation, and early defoliation. In severe cases, the young shoots of the grafts died back early in the season, as shown in Fig. 3. The greater the degree of incompatibility, the earlier the symptoms appeared. Thus, the leaves of the Common Plum grafted on Czar, those of St. Julien K grafted on Hale's Early Peach, of Victoria Plum grafted on Hale's Early Peach and of Quince F and Quince G grafted on Durondeau pear, began to turn yellowish and became markedly mottled as early as the end of June or in July. The leaves of Hale's Early Peach grafted on St. Julien K or Myrobolan B, turned pinkish-red and became curled about the middle of August. Durondeau and Conference pears worked on Quince F and Quince G also showed a yellowish-red colour in the leaves one month earlier than those worked on Quince A.

Whilst it is impossible to secure numerical data concerning these observations, Fig. 3 illustrates well the withering effect and dying back of the young growths and the discoloration of the basal leaves in an incompatible plum combination.

The flower buds of Hale's Early Peach on St. Julien K or on Myrobolan B were prominent the first season after grafting and appeared early in the autumn.

FIG 1   Hale's Early Peach budded on St Julien A (left), St Julien K (centre, incompatible) and St Julien C (right).

(Photographed   May 26th, 1936)
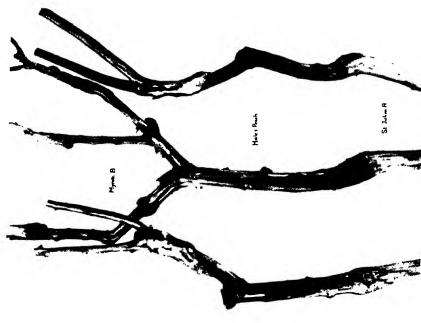
FIG. 2.   St. Julien C, K, and A (left to right) grafted on Hale's Early Peach.   St Julien K on Hale's Early is incompatible (centre).

(Photographed : May 26th, 1936.)

FIG. 4. Gum-formation in Hale's Early Peach. Peach stem grafted on St. Julien A. when grafted itself with Myrobolan B.

Note the white healthy wood of the topmost scion—Myrob B, and the under stock—St. Julien A. The black tissue of the intermediate Hale's Early is filled with gum.

FIG. 3. Two examples of Common Plum on Czar showing withering and dying back of young shoots.
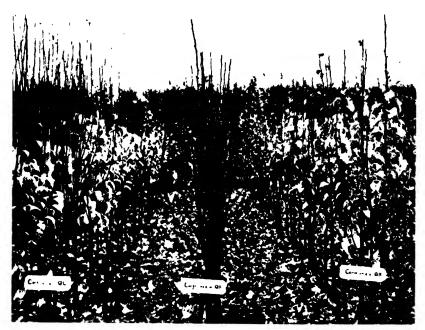
(Photographed : June 27th, 1936, three months after grafting.)

FIG. 5. Early defoliation of Conference Pear on Quince F (centre)
(Photographed November 2nd, 1936.)

FIG. 6. Early defoliation of Durondeau Pear on Quince F (left).
(Photographed. November 2nd, 1936.)

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With reciprocal grafts of Myrobolan B and Hale's Early, the leaves fell early in the autumn, and by the beginning of the following March all the wood was dry and dead. After examining these dead trees, masses of gum were observed in Hale's Early Peach, whether used as stock or scion, but there were none in the wood of Myrobolan B. This is illustrated in Fig. 4.

The incompatible combinations generally showed earlier defoliation as shown in Table VIII.

TABLE VIII.

Percentage of Defoliation of One-year-Grafts.

(Recorded October 31st, 1936.)

	Combinations.	Condition of Foliage.	% Defolia-tion.
Pears and Quinces.	Durondeau on Quince A	Dull purplish-green, tip defoliating	30
	Durondeau on Quince G	Ditto	40
	Quince A on Durondeau	Pale yellowish, leathery dry	20
	Quince G on Durondeau	Pale yellowish, dry curling	35
	Quince A on Quince A	Green, still growing	10
	Quince G on Quince G	Ditto	10
Plums and Plum Stocks.	Czar on Myrobolan B	Top leaves green, base defoliating	80
	Czar on Common Plum	Dull green, spotted, tip defoliating	90
	Myrobolan B on Czar	Green, spotted	20
	Common Plum on Czar	Yellowish, dry, tip defoliating	95
	Myrobolan B on Myrobolan B	Green, still growing	10
	Common Plum on Common Plum	Green, just stopped growing	15
Peaches and Plum Stocks.	Hale's on Peach G37/9*	Green, still growing	10
	Hale's on Brompton	Green, lower leaves pinkish	20
	Hale's on Myrobolan B	Few leaves left, reddish, flower buds prominent	99
	Hale's on St. Julien A	Green, lower leaves pinkish	50
	Hale's on St. Julien K	All defoliated, dry wood	100
	St. Julien A on Hale's	Top leaves green, lower leaves yellowish	70
	St. Julien K on Hale's	Yellowish, mottled, dry wood	90
	St. Julien A on St. Julien A	Green, lower leaves yellowish	50
	St. Julien K on St. Julien K	Green, still growing	40

* Peach G37/9, vegetatively raised clonal selection of seedling peach stocks.

Apart from Czar on Myrobolan B and the reciprocal combinations of Hale's Early Peach and St. Julien A (in which early leaf fall is a varietal characteristic) it will be seen that a much greater proportion of the foliage had dropped by October 31st, from the incompatible than from the compatible combinations.

Moreover, the symptoms of earlier defoliation, etc., were more pronounced on the older trees of the second year's growth. Figs. 5 and 6 show the uniformly defoliating trees of Conference and Durondeau pears worked on three selections of quince stocks, and the earlier defoliation is very obvious with Conference and Durondeau on Quince F stocks.

Finally, it was observed that in all incompatible combinations, the leaves at the top of the branch fell first, whereas in all compatible combinations, these

leaves usually remained attached for a long period after the lower leaves had fallen.

3. *Gradual Decline of Annual Wood Growth.*

A comparative study was made of the annual wood growth of compatible and incompatible combinations.   The trees were planted in replicated plots and had been uniformly treated since planting.   All the trees in each plot were measured and averaged and the results are given in Tables IX-XI.

TABLE IX.

Annual Wood Growth.   Pears on Quinces.

Combinations.	Number and age of trees measured.					
	26-39 one-year trees.		29-40 two-year trees.		10 three-year trees.	
	cm.	S.E.	cm.	S.E.	cm.	S.E.
Conference on Quince A ..	69	2·9	407	12·8	334	14·3
Conference on Quince C ..	81	2·8	348	9·4	—	—
Conference on Quince F ..	75	3·0	385	11·6	216	18·0 ·
Durondeau on Quince A ..	79	3·0	559	20·7	529	37·4
Durondeau on Quince C ..	94	2·9	561	21·1	—	—
Durondeau on Quince F ..	50	3·0	117	6·9	117	14·3

TABLE X.

Annual Wood Growth.   Plums on Plum Stocks.

Combinations.	Number and age of trees measured.			
	25-39 one-year trees.		33-39 two-year trees.	
	cm.	S.E.	cm.	S.E.
Victoria on Myrobolan B	223	6·3	714	18·0
Victoria on Common Plum	173	5·7	444	19·4
Czar on Myrobolan B	219	7·1	836	24·4
Czar on Common Plum	88	6·6	254	23·6

TABLE XI.

Annual Wood Growth.   Peaches on Plum Stocks.

Combinations.	6-19 one-year trees.	
	cm.	S.E.
Hale's Early on Peach G37/5	1,543	227·9
Hale's Early on Peach G37/9	1,025	135·1
Hale's Early on Brompton	898	86·8
Hale's Early on Myrobolan B	187	26·0
Hale's Early on St. Julien A	836	40·4
Hale's Early on St. Julien C	678	27·1
Hale's Early on St. Julien K	46	15·4

Among the pears on quince stocks, Conference showed more new wood growth on Quince F than on Quince A in the first year, but there was a gradual decrease in the second and third years. On the other hand, Durondeau made slightly less growth in the first year on Quince F than on Quince A, and showed a very rapid decline in the second and third years.

Among the plums, Czar had made much less wood growth by the end of the first season on Common Plum than on Myrobolan B. In the second year the difference was even more pronounced, the incompatible combination producing
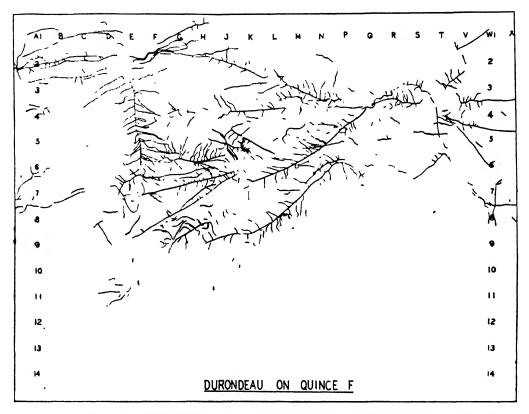
FIG. 7. Pear—Durondeau on Quince F. Showing extension and depth of new root growths as observed in Root Observation Box—1936.

only one-quarter the length of wood formed by the compatible one. Victoria had made slightly less growth in the first year on Common Plum than on Myrobolan B, but the difference in the second year was in about the same proportion. This was probably due to the normal influence of the Common Plum as a dwarfing stock.

With peaches, one-year-old trees only were available, but the difference in wood growth was very great. Hale's Early had made less than one-twentieth of the amount of growth on St. Julien K than on St. Julien A. It had made only about one-fifth of the amount of growth on Myrobolan B that it made on

Brompton.   Hale's Early on Brompton and on St. Julien A made about equal amounts.   Hale's Early on peach stock G37/9 made the greatest amount of wood during the end of the first season, and from the appearance of the foliage, it looked the healthiest of all the combinations.

As with the symptoms previously described, the more incompatible the combination the more rapid the decline in the annual wood growth.

An interesting phenomenon was observed in this connection, namely that all the incompatible combinations actually made more wood growth than the
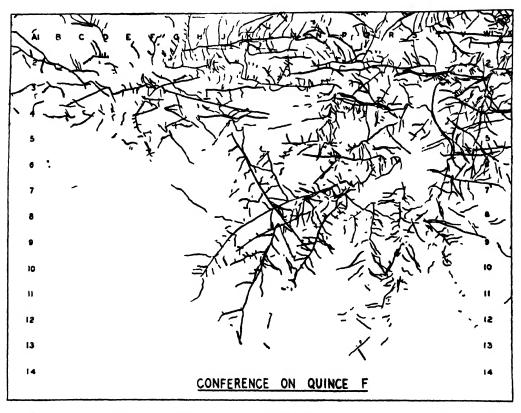
FIG. 8.   Pear—Conference on Quince F.   Showing extension and depth of new root growths as observed in Root Observation Box—1936.

compatible combinations in the initial stage.   In all cases, however, it was followed by a subsequent decline.   Tukey (62) observed the same thing with *Pyrus communis* varieties worked on *P. serotina*, *P. calleryana* and *P. ussuriensis*. This point will come out more clearly in the section dealing with scion/stock ratio.

4.   *Gradual decline of new root growth.*

Observations and measurements of new root growth were made by the root observation box method designed by Rogers (53).   Two-year-old trees

approximately equal in size were planted in root observation boxes with windows on their north and south sides. The soil (half fine quartz sand and half loam) was mixed and steam-sterilized before being put into the boxes. The trees were root pruned uniformly, weighed, and planted in November 1935.

Owing to the limited number of observation boxes and the enormous amount of labour entailed in recording new root growths, only two scion varieties and two stocks in each group were chosen. However, the arrangement of planting was designed to facilitate observation of one variety on a compatible stock

DURONDEAU ON QUINCE A
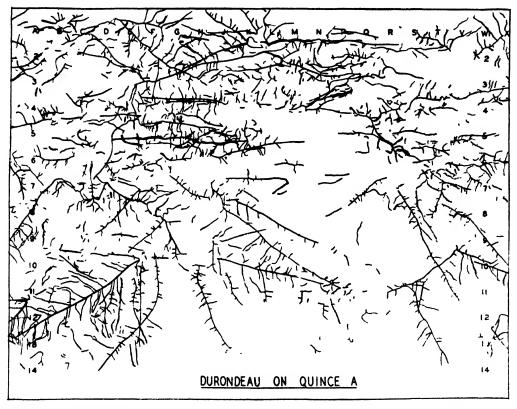
FIG. 9. Pear—Durondeau on Quince A. Showing extension and depth of new root growths as observed in Root Observation Box—1936.

(e.g. Durondeau on Quince A) and the same variety on an incompatible stock (e.g. Durondeau on Quince F). It was also possible to make comparisons with another combination which is supposed to have a much delayed occurrence of incompatibility, such as Conference on Quince F. With this arrangement it was therefore possible to observe the growth of the same scion variety on compatible and incompatible stocks, and also the root growths of the same stock variety carrying two different scion varieties.

Only one variety of peach, Hale's Early, was tested on compatible and incompatible St. Julien selections. In order to ascertain whether there was any

difference in the vigour and period of root growths between these two St. Julien selections, unworked stocks were planted side by side in the box separated by glass in the middle, and the new root growths of each were recorded and compared.

Two trees of one of the following combinations were planted in each root box :

> Conference on Quince F.
> Durondeau on Quince F.
> Durondeau on Quince A.
> Czar on Myrobolan B.
> Czar on Common Plum.
> Victoria on Common Plum.
> Hale's Early Peach on St. Julien A.
> Hale's Early Peach on St. Julien K.
> St. Julien A (unworked).
> St. Julien K (unworked).

In order to hasten the symptoms of incompatibility no fertilizers were applied, but equal amounts of water were given during very dry days in the summer.

All new root growths which appeared through the glass were carefully drawn every fortnight on root charts. Up to the time of cessation of root growth in the winter of 1936 the increments in length of the already visible roots and the lengths of new roots developed during each fortnightly period were recorded and added together. Three of the actual root charts of pears on quince stocks are reproduced in Figs. 7, 8 and 9.

Table XII shows the total number of new roots and the total lengths of new root growth for each combination of two trees from two windows as recorded from April 7th to November 3rd, 1936.

TABLE XII.

New Root Growth of Pear, Plum and Peach Combinations.

Combinations.				Total No. of New Roots.	Total Lengths (cm.).
Durondeau on Quince A	..	..	..	4,160	14,755
Durondeau on Quince F	..	..	..	1,812	6,999
Conference on Quince F	..	..	..	3,335	14,282
Czar on Myrobolan B	..	..	..	3,727	16,842
Czar on Common Plum	..	..	..	2,383	9,082
Victoria on Common Plum	..	..	..	2,694	11,384
Hale's Early on St. Julien A	..	..		1,662	5,956
Hale's Early on St. Julien K	..	..		12	36
St. Julien A (unworked)*	..	..	..	1,207	5,106
St. Julien K (unworked)*	..	..	..	1,612	6,509

* Only one tree planted in each box.

It will be seen that the incompatible trees had a smaller number and a smaller total length of new roots than the compatibles. For instance, the compatible combination Durondeau on Quince A produced more than double the total length of new roots in one season's growth than the incompatible combination, Durondeau on Quince F. Likewise, Conference on Quince F (a much delayed incompatible combination) had developed more than double the total length of new roots in one season as compared with the more acute form of incompatible combination, Durondeau on Quince F. This fact probably indicates that the symptoms of incompatibility proceeded from the scion downwards. The greater the degree of incompatibility, the more rapid and more marked was the decline in root growth.

The root charts show that the extension of the new root growths of the incompatible combinations was very much limited both horizontally and vertically. For example, the new roots of Durondeau on Quince F and of Czar on Common Plum, were much shallower and more sparse than those on the corresponding compatible combinations.

Another very interesting point was that the first appearances of new root growths from the incompatible combinations occurred much later in the spring than those from the compatible combinations. The new roots of Durondeau on Quince F started to show themselves about six weeks later than those of Durondeau on Quince A. The former combination was also much later than the delayed incompatible combination Conference on Quince F. Hale's on St. Julien A started about four weeks later than St. Julien A unworked. The same phenomenon was observed again in the same boxes in the Spring of 1937. The root growth curves for pears and plums, recorded fortnightly, show the delayed growth of new roots on incompatible combinations (see Figs. 10 and 11).

Furthermore, it was noted that although St. Julien A and St. Julien K represent two extremes of compatibility and incompatibility for Hale's Early Peach, yet (as can be seen in Table XII) there was no significant difference on unworked stocks either in the number or the total length of the new roots.

The dates of termination of the new root growths for compatible and incompatible combinations were also compared. It was quite obvious that Durondeau on Quince F ceased to grow later in the season than Durondeau on Quince A. It would appear that these differences in the periods of starting and terminating root growth cannot be explained by scion influence alone, but that they are likely to be due to some form of incompatibility at the union.

It is often asked whether in an incompatible combination, the scion or the root dies first. By using this root box method it is hoped that some light may be shed on this problem. In the more severe cases of incompatibility (Durondeau on Quince F) root growth had decreased very considerably, whilst in the less acute case (Conference on Quince F) about the same amount of new root growth
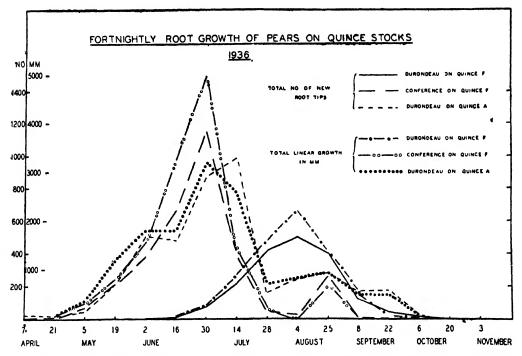
FIG. 10. Fortnightly Root Growth of Pears on Quince Stocks. Note the slow growth of the incompatible combination Durondeau on Quince F.
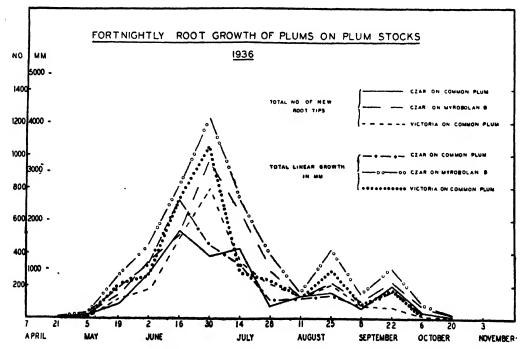
FIG. 11. Fortnightly Root Growth of Czar and Victoria Plums on Plum Stocks. Note the slow growth of the incompatible combination Czar on Common Plum.

had been maintained as in the compatible combination Durondeau on Quince A, although the new shoot growth of Conference on Quince F had decreased quite considerably at the time of recording. Moreover, observations in the field showed that from many incompatible combinations a strong bunch of suckers often arose from the stock when the scions were dead. Again, in many cases, the withering and dying back of the scions proceeded from the tops downwards, as previously described. It seems quite reasonable to think, therefore, that the scion declined first.

5. *Scion/stock ratio.*

In order to study more closely the relationship between stock and scion in incompatible combinations, a series of uniformly grown trees was carefully excavated by the trench method described by Rogers (52). The roots were washed as soon as excavated, and the trees were weighed as soon as they had become air-dry. Plans of their root systems were drawn.

Dry weights of pear combinations were also taken. Their scions and stocks were cut into short pieces and dried in an oven for five days at a temperature of 90°-100° C.

The scion/stock ratios obtained for pears, plums and peaches are presented in Tables XIII-XV.

<div align="center">TABLE XIII.</div>

Scion/Stock Ratio for Pears on Quinces. Average dry weight of Ten Two-year-old Trees for each Combination.

Combinations.				Mean Scion weight (gm.).	Mean Stock weight (gm.)	Scion/Stock Ratio.
Conference on Quince A	..	..	..	.. 176	123	1·47
Conference on Quince C	..	..	..	.. 184	164	1·13
Conference on Quince F	..	..	..	.. 183	102	1·80
Durondeau on Quince A	..	..	..	.. 143	118	1·23
Durondeau on Quince C	..	..	..	.. 180	132	1·37
Durondeau on Quince F	..	..	..	.. 33	56	0·58
Pitmaston on Quince A	..	..	..	.. 176	126	1·39
Pitmaston on Quince C	..	..	..	.. 154	126	1·20
Pitmaston on Quince F	..	..	.	.. 183	128	1·43

Gen. Mean = 1·29
S.E. = 0·06
Sig. Diff. = 0·18

With Conference, the scion/stock ratio was largest on Quince F. There were significant differences between all stocks in the order of magnitude of Quince F, A, and C.

With Durondeau, the scion/stock ratio was smallest on Quince F. The ratios for the trees on Quince A and Quince C were not significantly different from each other, but the ratio for that on Quince F was significantly smaller than that for those on Quince A and C.

There was no marked difference with Pitmaston Duchess on the three stocks.

In addition there were significant differences when Conference, Durondeau and Pitmaston on all stocks were compared, and also when the three rootstocks were compared worked with all scions.

The results obtained with plums fully confirm those obtained with pears. Czar worked on the incompatible Common Plum stock had a much lower scion/stock ratio than Czar on the compatible Myrobolan B. Victoria, which grows healthily on both of these stocks, showed the same kind of effect, but to a much less degree. The difference between stocks showed a highly significant effect. There were again significant differences between the two varieties.

TABLE XIV.

Scion/Stock Ratio for Plums on Plum Stocks. Average dry weight of Fifteen Two-year-old Trees for each Combination.

Combinations.				Mean Scion weight (gm.).	Mean Stock weight (gm.).	Scion/Stock Ratio.
Czar on Myrobolan B	..	..	..	1,286	527	2·35
Czar on Common Plum	..	..	..	273	190	1·40
Victoria on Myrobolan B	..	..	..	838	482	1·86
Victoria on Common Plum	..	..	..	455	325	1·40

Gen. Mean = 1·75
S.E.   = 0·08
Sig. Diff. = 0·23

Table XV shows that Hale's Early Peach on the incompatible Myrobolan B stock was significantly different in scion/stock ratio from the same variety on three compatible stocks. The differences between G37/5 and G37/9 and Brompton stocks, all compatible with Hale's Early Peach, were not significant.

An initially higher, but subsequently lower, scion/stock ratio was observed both on Hale's Early Peach on Myrobolan B and on the reciprocal combination, but in the former it showed up much earlier in the season. From bud break in the spring until about the middle of July, all the trees of Hale's Early grown on Myrobolan B made much quicker and stronger top growth as compared with those worked on Brompton or on peach stocks G37/5 and G37/9 ; but after the middle of July, the growth of Hale's Early on Myrobolan B began to drop, with

the occurrence of other symptoms, like drying, curling and premature colora-
tion of the leaves.   Durondeau Pear on Quince G, Victoria Plum on Hale's Early
Peach, Czar Plum on Common Plum and other incompatible combinations showed
the same phenomena.   Fig. 12 shows the growth of scion and stock of Durondeau
pear on Quince F, A, and C combinations reconstructed after excavation.

III.   THE IMMEDIATE CAUSES OF DECLINE AND PREMATURE DEATH IN INCOM-PATIBLE COMBINATIONS.

It is fairly common in the nursery for grafted or budded trees of incompatible
combinations to break at the union.   It is also not uncommon to see big trees in
the orchard (after eight or ten years of growth) suddenly blown down by a gale,
broken clean across at the union.   Such disasters suggest that incompatible
combinations are not firmly united.   Moreover, the external symptoms of
incompatible combinations, such as withering and early defoliation, gradual

TABLE XV.

*Scion/Stock Ratio for Peaches on Plum and Peach Stocks.   Average dry weight of
Seven One-year-old Trees for each Combination.*

Combinations.				Mean Scion weight (gm.).	Mean Stock weight (gm.).	Scion/Stock Ratio.
Hale's Early on Peach G37/5	..	..	..	544	430	1·28
Hale's Early on Peach G37/9	..	..	..	303	259	1·18
Hale's Early on Brompton	..	..	..	345	268	1·29
Hale's Early on Myrobolan B	..	..	..	51	89	0·58

Gen. Mean = 1·08
S.E.   = 0·08
Sig. Diff. = 0·25

decline in wood and root growth, and the dying back of the scion irrespective of
the still living and suckering stock, etc., already described, suggested that there
might be some form of obstruction to water or food transport at the union.
The following experiments were therefore designed to study in detail these
features of the defective union in incompatible combinations, for they may be
the immediate causes of the gradual decline and premature death of the tree.

1.   *Mechanical weakness of the union.*

Budded and grafted trees were examined in the field and laboratory to
ascertain whether the incompatible combinations had any form of abnormality
or weakness in the structure at the union.

(a) *Wood and bark discontinuity*. Trees were examined both externally, and with the bark peeled off at the union.   Three forms of discontinuity of wood and bark were observed, namely :

(i) Bark continuous, but wood discontinuous ; observed in Czar on Common Plum.

(ii) Bark discontinuous but wood continuous ; observed in Durondeau on Quince A.

(iii) Both bark and wood discontinuous ; observed in Durondeau on Quince F.

Figs. 13 and 14 show clearly these three forms of discontinuity.

In the pear, Dr. Jules Guyot worked on C_2 (a *Pyrus communis* seedling selection), both the bark and wood were very smooth and continuous (Fig. 13). Durondeau on Quince A showed almost smooth bark but a shallow crevice in the outside cortical region, whereas the wood fibres were smooth and continuous. Durondeau on Quince F showed a deep crevice in the bark, and a thick layer of " corky " cells in the wood at the union.

In the plum series, Czar on Myrobolan B showed continuity in bark and wood (Fig. 14), whereas both Czar on Common Plum and Oullin's Golden Gage on Myrobolan B (an exceptional known case of incompatibility) had a very distinctive crevice in the wood, at the union, although this was not externally visible.

These distinctive groups of incompatibility as shown by bark and wood discontinuity would seem to be directly correlated with a high degree of incompatibility.   The earliest symptoms of decline appeared in combinations in which both bark and wood were discontinuous.   On the other hand, much delayed symptoms of decline occurred in combinations in which only the bark or only the wood showed discontinuity.   Possibly this crevice at the union hinders or lessens the upward and downward transport of water, soil solution and manufactured food, thus causing a weaker growth of shoot and root.

(b) *Parenchymatous cell accumulations*.   Many strikingly incompatible grafts showed an accumulation of parenchymatous tissue at the union, visible to the naked eye.   In order to obtain a more distinctive contrast between stock and scion tissues the purple-tissued species of apple (*Pyrus Niedzwetzkyana* Hemsley) was used, both as stock and scion, in combination with apple, quince and pear varieties.   Some of these combinations resulted in unexpectedly vigorous growth.   *Pyrus Niedzwetzkyana* on Quince A, for example, formed shoots 3-4 feet long by the end of the season, but when longitudinal cuts were made
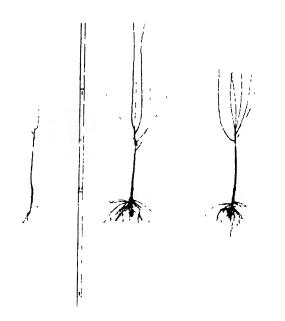
FIG. 12   Excavated Pears - Durondeau on Quince F (left), on Quince A (centre), and
on Quince C (right), showing root and shoot growth   Two year-old trees

Durondeau
on Quince F.

Durondeau
on Quince A.

Dr. G. Jules
on Pear C₂.
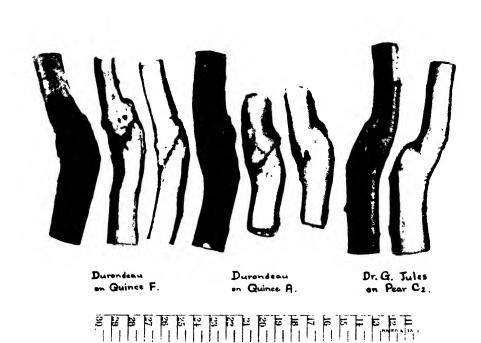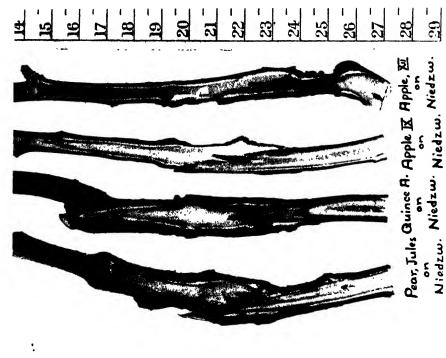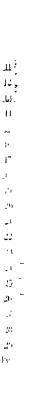
FIG. 13   Bark and Wood Discontinuity—Pears   Durondeau on Quince F (left), both
bark and wood discontinuous   Durondeau on Quince A (centre), both bark and wood
continuous, with slight crevice at the cortical region inside the bark.   Dr Jules Guyot on
Pear C₂ (right) is perfectly continuous.

14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

Pear, Jules Quince A. Apple IX Apple XII
on on
Niedzw. Niedzw. Niedzw. Niedzw.

FIG. 15. Pear, Dr Jules, Quince A. Apples No. IX and No. XII grafted on *Malus Niedzwetzkyana* stocks (One-year-old grafts.) Pear Dr. Jules and Quince A (two left) had heavy accumulation of "corky" cells Apples No. IX and XII had no accumulation although the colour of tissue remains distinct.

Czar on Czar on
Myrob B Common Plum

FIG 14 Bark and Wood Discontinuity—Plums (Czar on Myrob B has both bark and wood continuous (left) Czar on Com Plum has a continuous bark but discontinuous wood (right)

through the union, a thick layer of discoloured cells was visible between the purple tissue of the scion and the white tissue of the stock (Fig. 15).

On the other hand, with apple rootstock XII on *P Niedzwetzkyana* there was a distinct line of demarcation between the white and the purple tissue of scion and stock with no trace of discoloration, although this combination had made slightly less vigorous growth than had *P. Niedzwetzkyana* on Quince A. Fig. 15 shows the demarcation at the union in pear, quince, and apple grafts.
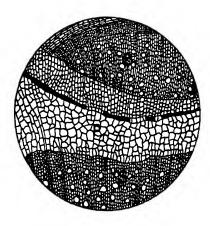
In order to follow the development of this parenchymatous tissue, the anatomy of the unions was studied in some 800 sections of inserted buds before these started to unfold. Plum and quince stocks were planted out in rows in the winter of 1935, on the randomized block system, and were budded in the summer of 1936 at random, within the stock rows, with varieties of plums, peaches, pears, and with the stocks themselves.

After budding, samples of the inserted buds were taken fortnightly, starting two weeks after insertion and continuing until April 1937. All the portions to be sectioned were fixed at once in Farmer's fixative, and then stored in 70 per cent. alcohol. Transverse sections 20μ thick were cut on a sliding microtome, and stained with safranin and Delafield's haematoxylin.
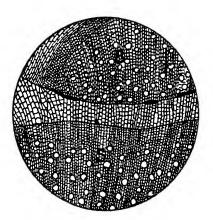
In the stock on stock combinations, such as Myrobolan B on Myrobolan B, newly developed cells between the bud and the adjoining stock were first observed about four weeks after budding. In the compatible scion-stock combinations, such as Czar on Myrobolan B, these cells were observed about six to eight weeks after budding, but in the incompatible combinations, such as Czar on Common Plum, the buds adhered very loosely to the stock and consequently were very difficult to cut, even twelve weeks after budding. Instead of growing together more or less uniformly the new cells at the union were much enlarged and were arranged in a markedly irregular way. This large-celled tissue, in which lacunae were sometimes present, was produced either by the stock, as in Hale's Early Peach on Myrobolan B, or by the bud, as in Czar on Common Plum. In later samples these irregular and loosely arranged cells were seen as masses of dead tissue. Both cross and longitudinal sections were examined, and both presented the same feature in compatible unions, viz. large-celled parenchyma and, later, dead cell accumulations as shown in Fig. 16. The pears on quince stocks did not exhibit these phenomena until much later in their course of growth. However, in every incompatible combination, these abnormal cell accumulations increased more and more as the bud growth advanced. | In the delayed form of incompatibility these abnormal cells were present to a much less extent in the earlier stages, but as the buds grew the accumulations became larger and more prominent. Fig. 16 shows diagrammatically the contrast in new tissue formation at the union between incompatible and compatible combinations as seen in the course of microscopical examinations.
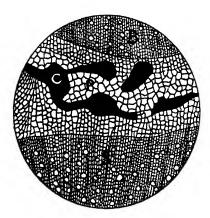
(c) *Ease of breaking at the union and nature of the break.* In order to see whether the presence of large-celled parenchyma and dead cell accumulations
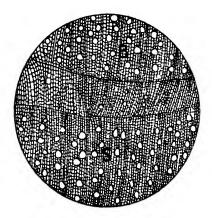
Sampled : September 15th.

Sampled : September 15th.

Sampled : November 15th.

Sampled : November 15th.

President on Common Plum.

Common Plum on Common Plum.

Fig. 16. Diagrams showing Transverse Sections of Bud-Unions. Plums—Budded August 4th, 1936.

B = Bud.
S = Stock.
P = Enlarged parenchymatous cells.
C = " Corky " dead cells.

would lead to mechanical weakness of the union, special apparatus was designed by the author to measure the force required to break grafted trees at the union. This is shown in Fig. 17.

Stems of compatible and incompatible combinations containing the piece of union were fixed and tightly held by strong clamps in the position shown in Fig. 17.

In this figure A is a fixed point.   The distance from B to C is ten times the distance from B to A, thus making a tenfold leverage.   An upward pull indicated as 1 lb. on the spring balance means a force of 10 lb. at the graft union.

A large spring balance was fastened at the end for pulling and reading. A wire-strainer was used so that before each pull the lever could be adjusted
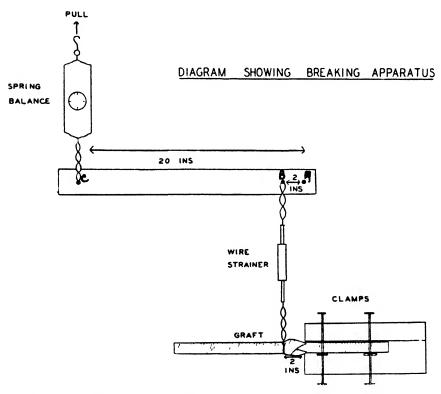
FIG. 17.   Diagram of Breaking Apparatus for Strength-at-Union Test.

exactly parallel with the graft union.   For the tests, six trees of pear on quince and plum on plum, and seven of peach on plum and peach, of uniform size, of each combination, were used.   The tests were made in the Spring of 1937.

It was noted that compatible unions of two-year-old pears, plums and peaches required a pull of as much as 700 lb. to break them.

The number of pounds required to break each union were calculated per square centimetre of the cross-sectional area of the union, and these are given in Tables XVI-XVIII.

All three varieties of pears, Conference, Durondeau and Pitmaston, when worked on Quince A, revealed a very significantly stronger union as compared with those worked on Quince F, except Conference on Quince F, which has a much delayed form of incompatibility.   The weakness of the union as expressed by this test seems to be in direct proportion to the degree of incompatibility.

TABLE XVI.

*Strength-at-Union Test of two-year-old Pears on Quince Stocks.**

Combinations.			Mean Diameter at Union (cm.).	Mean total lb. required to break.	Mean lb. to break per sq. cm. of cross-sectional area.
Conference on Quince A	..	..	3·41	199·5	21·7
Conference on Quince C	..	..	3·63	278·3	26·9
Conference on Quince F	..	..	3·53	214·3	21·9
Durondeau on Quince A	..	..	2·88	211·3	32·7
Durondeau on Quince C	..	..	2·84	150·0	23·6
Durondeau on Quince F	..	..	1·63	18·7	9·1
Pitmaston on Quince A	..	..	3·37	214·8	24·1
Pitmaston on Quince C	..	..	2·89	164·2	24·7
Pitmaston on Quince F	..	..	3·48	145·8	15·4

Gen. Mean = 22·2 lb. per sq. cm.
S.E.   =   1·23
Sig. Diff.   =   3·88

* A further series of twelve trees each of these pear and quince combinations have been tested since the above was written, and yielded exactly the same results.

TABLE XVII.

Strength-at-Union Test of two-year-old Plums on Plum Stocks.

Combinations.			Mean Diameter at Union (cm.).	Mean total lb. required to break.	Mean lb. to break per sq. cm. of cross-sectional area.
Czar on Myrobolan B	..	..	3·91	†685·7	†76·9
Czar on Common Plum	..	..	1·92	168·2	52·7
Victoria on Myrobolan B	..	..	3·33	515·4	59·9
Victoria on Common Plum		..	2·30	261·4	62·5

Czar on Myrobolan B : Czar on Common Plum.
S.E. difference between means =   8·8
Significant Difference   = 18·9
Victoria on Myrobolan B : Victoria on Common Plum.
S.E. difference between means =   5·8
Significant Difference   = 12·5

† Bent, but still unbroken.

TABLE XVIII.

Strength-at-Union Test of two-year-old Peaches on Plum and Peach Stocks.

Combinations.		Mean Diameter at Union (cm.).	Mean total lb. required to break.	Mean lb. to break per sq. cm. of cross-sectional area.
Hale's Early on Peach G37/5	..	2·64	393·7	73·7
Hale's Early on Brompton	..	2·47	302·3	63·1
Hale's Early on Myrobolan B	..	1·57	54·0	25·6

Hale's Early on Peach G37/5 . Hale's Early on Brompton.
S E. difference between means = 9·2
Significant Difference = 19·7
Hale's Early on Brompton : Hale's Early on Myrobolan B.
S.E. difference between means = 3·9
Significant Difference = 8·7

These three scion varieties when worked on Quince C gave rather variable results. The trees of Conference on Quince C had a comparatively strong union, whereas these of Durondeau and Pitmaston on Quince C had a somewhat less strong one. Moreover, among the same group of trees tested, such as Durondeau on Quince C, two trees out of six showed a relatively easy and clean break, whereas the four others showed fairly difficult and rough breaks. Pitmaston Duchess on Quince C also behaved in just the same way. The same thing was observed when another series, of twelve trees each, was tested in July 1937. This indicates that Quince C is probably a partially incompatible stock for Durondeau and Pitmaston Duchess pears, though this incompatibility is often indefinitely delayed.

With plums, most of the unions in trees of Czar on Myrobolan B could not be broken at pull of 700 lb. and they are very significantly stronger than the unions of Czar on Common Plum. There was no significant difference between the unions of Victoria on Myrobolan B and on Common Plum.

Again, with peaches, the incompatible Hale's Early had a very much weaker union on Myrobolan B than on Brompton. The difference between Hale's Early on Peach G37/5 and on Brompton is not significant.

It was noted that the force required to break the unions between Pitmaston Duchess pear and Quince F stock was much less than that necessary to break the unions between the same pear scion and Quince stocks A and C; although, as was shown in the scion/stock ratio study, this variety had made much more vigorous growth on Quince F than on either of the compatible stocks at the time of the breaking-test.

Furthermore, an examination of the broken surface of the incompatible unions, such as Durondeau on Quince F or Czar on Common Plum, revealed the presence of inwardly curved layers of cells produced by the stock and the scion,

such as occur in the healing of wounds.   This suggests that the union between the stock and scion formed in successive seasons had been incomplete.

It is obvious, therefore, that all incompatible combinations possessed relatively weak unions.   Moreover, in every case tested, they had invariably a clean and smooth break, as is clearly shown in Durondeau on Quince F in Fig. 18.   The same phenomenon was observed with Czar on Common Plum and Hale's Early Peach on Myrobolan B.   On the other hand, all the compatible unions showed a very rough and irregular break.   These differences are also shown in Fig. 18.

2. *Obstruction of water, soil nutrients and elaborated foods at the union.*

The symptoms of withering and premature autumn coloration of the foliage, together with those of premature flower bud formation and early defoliation, as already described, suggest that there may be a deficiency of moisture content in the scion due to obstruction at the union.   To determine whether lack of water existed in incompatible combinations above the union, the following experiment was carried out to show statistically the moisture content of the leaves and shoots.

Samples of leaves and current-year shoots were taken from compatible and incompatible combinations on September 18th, 1936.   Four samples were taken from four different trees of each combination.   The petioles were removed from the leaves before weighing.   Samples were also taken from unworked stocks to ascertain whether any difference existed between the so-called compatible and incompatible stocks.   Immediately after the shoots were sampled in the field, the fresh weights of the shoots and leaves were determined.   Then, all samples were put into an electric oven to dry for three days at a temperature of 90°-100° C. Dry weights were then determined and the moisture content of the different samples was calculated.   The results are given in Tables XIX, XX and XXI.

TABLE XIX.

Moisture Content of Pears on Quinces.

Combinations.		% of moisture of one-year trees.			% of moisture of two-year trees.			
		Stems.		Leaves.		Stems.		Leaves.
			S.E.		S.E.		S.E.		S.E.
Durondeau on Quince A	..	55·8	1·1	60·4	0·2	51·5	0·4	57·6	0·4
Durondeau on Quince F	..	50·8	0·5	55·9	0·8	43·7	0·2	48·5	0·4
Conference on Quince A	..	56·1	0·5	62·8	0·3	54·5	0·3	56·6	0·2
Conference on Quince F	..	51·4	0·4	57·8	0·4	50·9	0·3	57·2	0·3
Quince A (unworked)		58·4	0·2	61·1	0·1	—		—
Quince F (unworked)		60·2	0·4	61·9	0·3	—		—

FIG. 19 Dye Injection of Pears. Durondeau on Quince A (left). Durondeau on Quince F (right). Injected July 1936, with 0.2 per cent Alkali Blue.
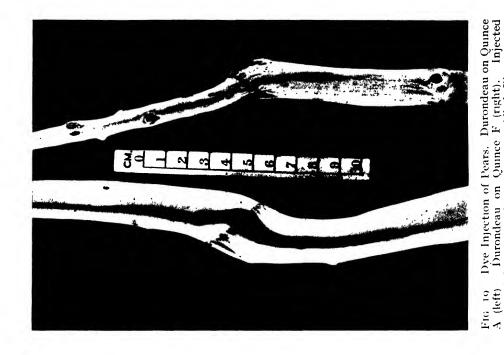

FIG. 18 Strength-at-Union Test.—Durondeau on Quince A, F, and C. Durondeau on Quince F (centre) shows a very clean and smooth fracture. Durondeau on Quince C (right) had a few trees with a clean but most of them with a rough fracture. Durondeau on Quince A (left) had a rough, tearing break.

TABLE XX.

Moisture Content of Plums on Plum Stocks.

Combinations.						% of moisture of two-year trees			
						Stems.		Leaves.	
							S.E.		S.E.
Czar on Myrobolan B	..	..	..	..	..	58·5	0·1	70·6	0·2
Czar on Common Plum	..	..	..	..	..	55·0	0·4	69·1	0·4
Victoria on Myrobolan B	..	..	..	..	..	53·0	0·4	67·9	0·6
Victoria on Common Plum	..	..	..	..	..	51·9	0·4	69·8	0·3
Myrobolan B (unworked)	..	..	..	..	..	60·1	0·9	67·0	0·7
Common Plum (unworked)	..	..	..	..	..	58·5	0·9	66·8	0·7

TABLE XXI.

Moisture Content of Peaches on Plum Stocks.

Combinations.						% of moisture of one-year trees.			
						Stems		Leaves.	
							S.E.		S.E.
Hale's Early on Peach G37/5	..	..	..	..	..	61·2	0·9	70·4	0·2
Hale's Early on Brompton	..	..	..	..	..	62·5	1·2	69·9	0·5
Hale's Early on Myrobolan B	..	..	..	..	..	57·2	0·5	58·5	0·4
Hale's Early on St Julien A	..	..	..	..	..	63·7	0·4	69·5	0·6
Hale's Early on St. Julien K	..	..	..	..	..	45·0	0·4	55·2	0·2
Peach G37/5 (unworked)	..	..	..	..	..	62·5	0·2	71·9	0·3
Brompton (unworked)	..	..	..	..	..	64·7	0·6	70·8	0·2
Myrobolan B (unworked)	..	..	..	..	..	60·2	0·9	67·0	0·7
St. Julien A (unworked)	..	..	..	..	..	55·6	0·6	68·7	0·1
St. Julien K (unworked)	..	..	..	..	..	55·5	0·5	70·3	0·5

In the same comparable group, Durondeau on Quince F and Conference on Quince F were much lower in moisture content of the shoots and leaves than were the same varieties on Quince A, except in one case of leaves of the second-year-Conference on Quince F. The shoots and leaves of Czar on Common Plum were also drier (especially the shoots) than those of Czar on Myrobolan B, whereas those of Victoria showed approximately the same moisture content on both stocks. Hale's Early Peach worked on St. Julien K and on Myrobolan B, two incompatible stocks for peaches, also had a much lower percentage of moisture in the shoots and leaves than did the same variety worked on Peach seedling selection G37/5, Brompton and St. Julien A, three compatible stocks for peaches.

There was very little difference in moisture content of the unworked stocks. St. Julien A and St. Julien K showed a slightly lower moisture content in the

shoots than the other unworked stocks, but the moisture content of the leaves was about the same.

The phenomenon, like the other symptoms noted above, was most marked in the more incompatible combinations, such as Hale's Early Peach on St. Julien K and Myrobolan B.

In the pear combinations, it was observed that the difference in moisture content between compatible and incompatible combinations increased in direct relation to the age of the tree, except in the leaves of Conference on Quince F, where a slight increase over Conference on Quince A was recorded. This difference may have been due in part to variations in the kinds of tissue in the samples tested. It should be noted, however, that there was practically no difference in moisture content between unworked stocks, especially in the leaves, in spite of the variation in size and morphology of the leaves and stems of those stocks.

The samples were taken at a time when the transpiration rate was high and the trees were still fairly active in growth. It is therefore reasonable to suppose that these differences in moisture content in incompatible combinations may indicate either a partial obstruction to the passage of moisture at the union, or a decline of the root absorbing surface.

In order to determine whether there is any definite form of obstruction to the passage of water or soil solutions or to the transference of food reserves from one partner to the other at the union, a further series of experiments was carried out as follows:

(a) *Dye Injection to show obstruction at the union.* Dyes were injected into the stems of growing trees in July 1936. Small holes were bored with a special drill to about ¼ inch in depth both 2 inches above and 2 inches below the union. The portion of the bark at the union was peeled off to observe the ascending and descending flow of dyes. The dyes used were Alkali Blue (0·2%), Water Blue (0·2%), Ponceau Red (0·2%), and Acid Fuchsin (0·2%).

The passage of the dyes was traced after injection to see how long they took to pass the union. It was discovered in preliminary tests that Acid Fuchsin and Ponceau Red travelled too quickly to make accurate measurements possible, Water Blue and Alkali Blue were therefore used in the comparable series. Alkali Blue travelled much more slowly than Water Blue. The dyes were injected by connecting glass tubes with small rubber ones as suggested by Roach (51).

At that time of the year it was found that the upward movement of the dyes was much more rapid and also much greater in volume than the downward movement.

The average results of the Alkali Blue injections of three two-year-old trees each are given in Table XXII.

TABLE XXII.

Results of Alkali Blue Injections in Pears and Plums.

Combinations.				Time required for dye to pass the union after injection.	Amount of dye absorbed 5 hours after injection.
Durondeau on Quince A	..	..	..	5 hours	20 c.c.
Durondeau on Quince F	..	..	..	18 ,,	6 ,,
Czar on Myrobolan B ..	..	..	..	14 ,,	28 ,,
Czar on Common Plum	..	..	..	24 ,,	15 ,,

Water Blue injections showed similar variation between the compatible and incompatible combinations, but the liquid was absorbed at a much more rapid rate and in greater quantity. This is probably due to the fact that Water Blue is a di- or tri-sulphonic Blue and is more soluble than the mono-sulphonic Alkali Blue. With plums there was practically no difference between Czar on Myrobolan B and Czar on Common Plum in the rate of ascending flow of Water Blue solution.

In incompatible combinations of pears there was a very limited flow of dyes and their paths were much contorted after passing through the union. This indicates that the wood vessels just above the union were contorted and obstructed. On the other hand, Durondeau on Quince A had a very copious and free flow of dye through the union, as shown in Fig. 19.

(b) *Water Conductivity Test to show obstruction at the union.* It had been observed that the incompatible grafts had much smaller leaves as compared with the compatible combinations. This smaller leaf-area persisted every season after grafting. On many highly incompatible grafts the young shoots and leaves died back, usually in the hot summer days of July and August, as shown in Fig. 3. Moreover, the moisture-content determinations of the leaves and young shoots, previously described, showed that there is a definite deficiency of water in the scion leaves and shoots of the incompatible combinations. Hence an experiment on water conductivity through the union was designed, to ascertain whether there was any obstruction at the union to the upward flow of water.

Water conductivity in branches of trees has been studied by Farmer (11), Rivett (49), and many others. Farmer showed that there was great variation between evergreen and deciduous trees, although the variability in each case was very high. Further, Langdon (37) and Holmes (26), described a kind of correlation between wood structure and water conductivity, but the method they employed, involving positive pressure and counting the drops, was too slow to be used on grafts. A negative pressure apparatus was therefore devised by

the author which permits the testing of six grafts at a time.   Readings can be made at intervals on six burettes connected to the six grafts, as shown in Fig. 20.

The pear and plum trees used in the experiment were two years old and the peaches one year old.   These were freshly dug, and in order to avoid the entrance of excessive air into the vessels, the parts containing the unions were cut under water into 8 cm. lengths (4 cm. above and 4 cm. below the middle of the union). A negative pressure of 735 cm. of mercury was maintained throughout the experiment with a ¼ horse power motor.   Fifteen minutes after the motor
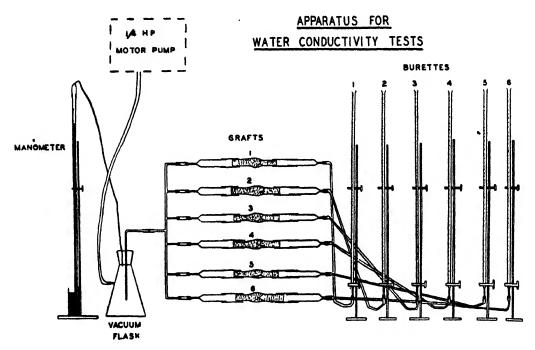
FIG. 20. Diagram of Apparatus for Water Conductivity Test.

started, readings were begun and they were repeated at intervals of ten minutes for one hour.

The total amount of water that passed through the union was calculated in cubic centimetres per sq. cm. of the cross-sectional area, by taking the average of the diameters of the scion and stock, measured 1 cm. above and below the union.   Tables XXIII, XXIV and XXV show the results of the experiment, the figures being the average of six trees for each combination.

In every case the incompatible combinations showed a much lower efficiency in conductivity.   Durondeau on Quince F passed only one-third of the amount of water that Durondeau on Quince A passed.   Czar on Common Plum passed only about one-tenth of the amount of water that Czar on Myrobolan B passed.

TABLE XXIII.

Water Conductivity Test through the Union—Pears on Quince Stocks.

Combinations.			Mean cross section area (sq. cm.).	Mean total water passed in 1 hour (c.c.).	Mean c.c. of water passed per hour per sq. cm.
Conference on Quince A	..	..	8·60	313·9	37·3
Conference on Quince C	..	..	7·56	305·6	40·4
Conference on Quince F	..	..	7·35	328·5	44·5
Durondeau on Quince A	..	..	5·02	133·5	25·4
Durondeau on Quince C	..	..	4·06	109·1	26·8
Durondeau on Quince F	..	..	2·97	26·2	8·8
Pitmaston on Quince A	..	..	5·74	335·9	58·1
Pitmaston on Quince C	..	..	4·61	134·9	28·1
Pitmaston on Quince F	..	..	6·11	157·0	25·0

Gen. Mean = 32·7 c.c. per hour per sq. cm.
S.E. = 2·01
Sig. Diff. = 6·32

TABLE XXIV.

Water Conductivity Test through the Union—Plums on Plum Stocks.

Combinations.			Mean cross section area (sq. cm.).	Mean total water passed in 1 hour (c.c.).	Mean c.c. of water passed per hour per sq. cm.
Czar on Myrobolan B	..	..	6·68	734·6	113·8
Czar on Common Plum	..	..	3·32	30·6	9·2
Victoria on Myrobolan B	..	..	6·43	703·9	109·3
Victoria on Common Plum	..		3·84	283·9	76·2

Gen. Mean = 76·2 c.c. per hour per sq. cm.
S.E. = 5·12
Sig. Diff. = 15·0

TABLE XXV.

Water Conductivity Test through the Union—Peaches on Plum and Peach Stocks.

Combinations.			Mean cross section area (sq. cm.).	Mean total water passed in 1 hour (c.c.).	Mean c.c. of water passed per hour per sq. cm.
Hale's Early on Peach G37/9	..		2·80	159·1	53·0
Hale's Early on Brompton	..	..	4·08	67·7	16·1
Hale's Early on Myrobolan B	..		1·64	4·3	2·6

Gen. Mean = 23·9 c.c. per hour per sq. cm.
S.E. = 7·12
Sig. Diff. = 22·4

The variation between Hale's Early Peach on Myrobolan B and on Peach G37/9 is even more striking.

Pieces of scion stems taken just above the unions and cut into 8 cm. lengths were also tested in a similar way. It was observed that with pears and plums there was practically no variation between scions that had been worked on compatible and incompatible stocks. Hale's Early Peach on Myrobolan B passed a much smaller amount of water than did Hale's Early on Peach G37/9. After splitting the pieces of scion it was actually found that there were many gum deposits in the vessels of the Hale's scion worked on the Myrobolan B stock. It seems likely that this gum materially lowered the conductivity.

On the budded trees most of the stock stems were too short to allow of a conductivity test. However, 8 cm. stem pieces of the unworked two-year stocks were tested in a similar way, and no appreciable difference was observed between comparable groups.

In the data obtained on the conductivity of the unions, the variability among the six individual trees tested was rather high (Tables XXIII-XXV), but in considering the difference between compatible and incompatible combinations, the figures are still highly significant.
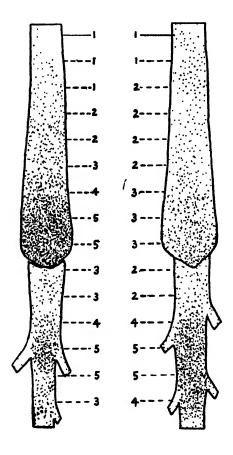
(c) *Examination at the union for starch and for mineral obstruction.* Experiments were made both on young incompatible scions, and on prematurely yellowing leaves by injecting nutrient solutions. The method used was that described by Roach (50), and the following nutrient solutions and their combinations were injected :

1. Urea, $CO(NH_2)_2$ .. .. .. 0·5%
2. Fe-citrate .. .. 0·05%
3. K_2HPO_4 .. .. 0·5%
4. K_2SO_4 .. .. 0·5%
5. H_2O .. .. control.

Injections by means of special tubes into young scions which showed the adverse effects due to incompatibility were made in the middle of July 1936 with St. Julien K on Hale's, Common Plum on Czar, and Quince G on Durondeau. No indications of recovery were obtained after injection. It was, however, possibly too late to have injected, as the scions were already showing severe symptoms of decline.

Leaf injections through small rubber tubes were made into ailing pear and peach leaves at 11 a.m. on July 8th and 9th, 1936. Peach leaves were somewhat too thin to hold the rubber tubes and these dropped off overnight. The yellow leaves of Durondeau on Quince F, however, showed a clear recovery, becoming dark green within seven days after injection with 0·05% ferric citrate.

Tests were also made in November 1935 on compatible and incompatible combinations to ascertain the variations in the amount of starch deposited above and below the union. Longitudinal sections 30μ thick were cut at 1 inch intervals along the stem, above and below the union of three Durondeau trees on Quince F and three Durondeau on Quince A. All the trees were two years old. The sections were then put into 0·5% iodine in potassium iodide solution.

Dur. on Quince F.   Dur. on Quince A.

FIG. 21. Diagram showing distribution of starch at the Union of Pears. (Two-year-old trees, November, 1935.) The figures 1-5 represent degrees of intensity of starch reaction at distances 1 inch apart.

There was a heavy deposit of starch just above the union in incompatible Durondeau on Quince F as indicated by the iodine test, whereas the portion of stem just below the union had very little starch. The compatible Durondeau on Quince A had a uniformly distributed amount of starch both above and below the union. This is illustrated in Fig. 21, which is a diagram prepared after assembling in order the 1 inch lengths that had been treated with iodine.

4

This observation appears to be in close agreement with observations made by Kostoff on grafts of certain Solanaceae (34, 35). The starch deposits above the union may possibly be an after-effect of the lack of water in the tissue or may be associated with the early maturity of the incompatible tree; but in considering the starch reaction just below the union, it is clear that there must have been some obstruction to the translocation of sugars and other soluble carbohydrates at the union.

IV. Possible Fundamental Causes of Incompatibility.

A common hypothesis held by many workers (14, 15, 16, 61) has been that the fundamental cause of incompatibility is the production of some form of toxin by one of the partners in a grafted or budded tree. However, amongst the deciduous fruit trees studied at East Malling, there were considerable differences in the occurrence and the degree of severity of incompatibility between a normal scion-stock and its reciprocal stock-scion combination. Moreover, Czar, a plum closely related to *P. domestica*, fails to grow on Common Plum (another *P. domestica* stock) whereas it grows very vigorously on Myrobolan B, which belongs to different species of Prunus, viz. *P. cerasifera*. Again, Oullin's Golden Gage, another variety of *P. domestica*, fails to grow at all on Myrobolan B, which is generally compatible with other plums. These observations seem to suggest that incompatibility is not likely to be due to the presence of toxic substances such as hydrogen cyanide or to poisonous alkaloids.

On the other hand, observations on the growth of different stocks in the field have often revealed great differences either in the period or in the amount of growth. For instance, Victoria Plum has a much shorter period of seasonal growth than Czar Plum. Likewise, unworked Common Plum stocks have a much shorter period of growth than Myrobolan B. These facts suggest strongly that the cause of incompatibility might be associated with some functional physiological differences in cambial activity, callusing, and other growth activities. Accordingly the following experiments were carried out to determine what effect, if any, the following physiological differences between stock and scion might have upon their compatibility.

1. *Different periods of cambial activity between stock and scion.*

Priestley (41) has shown that different plants vary in their seasonal periods of cambial activity, and has also described a stripping method (42) for studying the period of cambial activity. In accordance with this method strippings were made in the summer of 1936 on unworked stocks generally recognized as compatible and incompatible in each group and upon certain representative scion varieties. All the trees were growing in the same plot. A rectangular cut,

about 1 inch long and ⅛ inch wide, was made in the stock trunk. The bark was carefully peeled off and the strippings were collected.

It was observed that there was a difference in the stiffness of the bark while peeling at the beginning of June and also at the end of August among different two-year-old unworked stocks. Moreover, there was some variation in the thickness of the strippings at the beginning of June and at the end of August. It was found that the bark could not be peeled before June or after August. Table XXVI shows the condition of the bark and the thickness of the strippings during each period.

TABLE XXVI.

Condition of Bark-peeling and Strippings on Different Stocks.

Stocks.	June 1st.		July 1st.		August 1st.		August 28th.	
	Condi-tion of bark.	Thickness of strip-pings.	Condi-tion of bark.	Thickness of strip-pings	Condi-tion of bark	Thickness of strip-pings	Condi-tion of bark.	Thickness of strip-pings.
Quince A	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick
Quince C	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick
Quince F	Loose	Thinner	Loose	Thick	Loose	Thick	Loose	Thinner
Conference*	Loose	Thick	Loose	Thick	Loose	Thin	Loose	Thin
Durondeau	Loose	Thick	Loose	Very thick	Loose	Thick	Loose	Thick
Myrobolan B	Stiff	Thin	Loose	Thick	Loose	Thick	Stiff	Thick
Common Plum	Stiff	Thin	Loose	Thick	Loose	Thinner	Loose	Thin
Czar†	Stiff	Thin	Loose	Very thick	Loose	Thick	Loose	Thick
Victoria†	Stiff	Thin	Loose	Very thick	Loose	Thinner	Stiff	Thin
St. Julien A	Loose	Thin	Loose	Thick	Loose	Thick	Loose	Thick
St. Julien K	Stiff	Thin	Loose	Thinner	Loose	Thinner	Stiff	Thin
Brompton	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick
Hale's Early‡	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick

* Grafted on Quince A.
† Grafted on Myrobolan B.
‡ Grafted on St. Julien A.

From Table XXVI it seems clear that in early summer and again in late August most of the scion varieties had thicker strippings than the stocks, and this would seem to indicate an earlier or quicker differentiation of the cambial cells. It is interesting to note that Conference Pear and Victoria Plum had thinner strippings than other Pear and Plum varieties, and in Victoria the bark was fairly stiff toward the end of August. This probably indicates an earlier cessation of cambial activity.

Among the unworked stocks studied, one interesting fact observed was that all the so-called incompatible stocks had either a later start or an earlier cessation of cambial activity, as indicated by the stiffness of the bark and the thickness of the strippings.

Bark condition and strippings were also studied on two-year-old compatible and incompatible combinations at the end of August. Both scion and stock just above and below the union were studied and the strippings compared. The results are shown in Table XXVII.

TABLE XXVII.

Condition of Bark-peeling and Strippings of Compatible and Incompatible Combinations.

Combinations.	Scion.		Stock.	
	Condition of bark.	Thickness of strippings.	Condition of bark.	Thickness of strippings.
Conference on Quince A ..	Loose	Thin	Loose	Thick
Conference on Quince F ..	Loose	Thin	Stiff	Thin
Durondeau on Quince A ..	Loose	Thick	Loose	Thick
Durondeau on Quince F ..	Loose	Thin	Very stiff	Thin
Czar on Myrobolan B ..	Loose	Thick	Loose	Thick
Czar on Common Plum ..	Loose	Thick	Loose	Very thin
Victoria on Myrobolan B ..	Loose	Thin	Loose	Thick
Victoria on Common Plum ..	Stiff	Thin	Loose	Thin
Hale's on Myrobolan B ..	Stiff	Thin	Very stiff	Very thin
Hale's on Brompton ..	Loose	Thick	Loose	Thick
Hale's on St. Julien A ..	Loose	Thick	Loose	Thick
Hale's on St. Julien K ..	Stiff	Thin	Very stiff	Very thin

It will be seen that the incompatible combinations, both scion and stock, had stiffer bark and thinner strippings than the compatible combinations. It is also interesting to note that in incompatible combinations, as in Durondeau on Quince F, the Quince F stock had much stiffer bark and thinner strippings than the scion Durondeau. The same phenomenon was observed on Czar on Common Plum, Hale's Peach on Myrobolan B and St. Julien K, and it seems to indicate that in the incompatible combinations the cambial activity of the stock is more or less independent of the cambial activity of the scion.

2. *Difference in period of callus growth between stock and scion.*

Callus growth has been studied in stock and scion parts after grafting. Both compatible and incompatible grafts of pears, peaches and cherries were made early in March 1936. In each case ordinary whip and tongue grafting was used.

Samples were taken weekly from each combination and examined under a dissecting microscope to observe the amount of callus growth both on stock and on scion. The regions and amount of callus growth in each sample were diagrammatically drawn to facilitate comparison, and are illustrated in Fig. 22.

It was observed that incompatible combinations showed greater variation in the amount of callus growth between stock and scion than the compatible ones. The amount of callus formed, however, is not always greater in the stock, as has been shown by Proebsting (44). The variation was particularly noticeable on very incompatible combinations, such as St. Julien K on Hale's Peach, *Prunus serrulata* on Mahaleb, and Quince F on Durondeau.

It was further noted that in incompatible combinations the position and area of the callus growth were somewhat irregular as between stock and scion. The callus cells appeared first in every case at the blunt or thick end of the slanting cut on the stock and scion ; but in the incompatible combinations, the callus cells did not extend their growth to the thin end of the slant. On the other hand, in the compatible combinations, stock and scion extended their respective callus growths and soon covered the thin ends. In incompatible combinations the new callus cells of the stock were thus unable to get into contact with those of the scion, and in many cases, these cells died and turned black. Fig. 22 shows the exact positions and areas of callus growths as they were seen in fresh samples.

This variation in the periods of callus growth of the stock and scion seems coincident with the cambial activity described in the previous section. For instance, St. Julien K had a later stage of cambial activity than Hale's Early Peach, as shown in the strippings. It was observed in the callus study of the grafts that St. Julien K had much less callus growth, especially at the tip, in comparison with Hale's Peach when used either as stock or scion.

3. *Variation in the periods of foliation and defoliation.*

Studies have been made to see whether there is any correlation between the time of leaf unfolding and leaf fall in stock and scion and the degree of incompatibility.

To obtain the required data, stocks in the same plot and under the same treatment were chosen. The date of breaking into leaf was recorded for each stock : the number of leaves were counted, and the areas of a sample of ten leaves from each terminal shoot were measured in the first month after bud-break.

Although there were great differences in the times of bud-break, it was not possible to correlate them with incompatibility. For instance, Hale's Peach and Myrobolan B broke into leaf earlier than Brompton or St. Julien. There

was practically no difference in the date of leaf unfolding between Quince A and Quince F, both of which were much earlier than pear varieties. Similarly, the defoliation dates of the stocks showed no correlation with incompatibility.
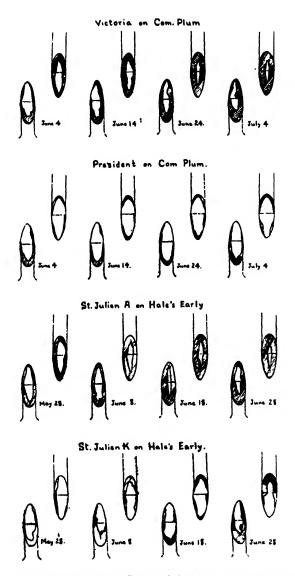

FIG. 22. Callus Growth of Compatible and Incompatible Grafts.

Shaded area—New callus.
Black area—Dead callus.

Thus, for example, Myrobolan B, a very late growing stock, has proved much more compatible with Czar Plum than has the early defoliating Common Plum stock.

When the date of bud-break and the rate of leaf-expansion were taken into consideration, it was noted that there was wide variation in the rate of leaf-expansion. For instance, Durondeau and Pitmaston pears expanded their leaves very much more slowly in the spring than Conference. Common Plum was about one month later in bud-break than was Myrobolan B, yet there was little difference between the two stocks in date of leaf-expansion. Similarly, Mahaleb F8/1/10, although showing bud-break about ten days later than Mazzard F5/1, expanded its leaves about ten days ahead of the Mazzard stock, as is shown in Table XXVIII.

TABLE XXVIII.

Dates of Bud-break and Leaf-expansion of Pears, Plums, Peaches, and Cherries—Two-year-old Stocks and two-year-old Scion Varieties.

(Recorded in 1936.)

Varieties.				Date of bud-break.	Date of full leaf-expansion.	Rate of leaf-expansion.
Durondeau on Quince A	..	..	..	.. March 20th	April 28th	Slow
Pitmaston on Quince A	..	..	..	.. March 15th	April 22nd	Slow
Conference on Quince A	..	..	..	.. March 10th	April 10th	Quick
Quince A (unworked)	..	..	..	.. February 20th	March 25th	Slow
Quince F (unworked)	..	..	..	.. February 15th	March 10th	Quick
Czar on Myrobolan B	..	..	..	.. March 10th	April 10th	Slow
President on Myrobolan B	..	..	..	.. March 10th	April 12th	Slow
Victoria on Myrobolan B	..	..	..	.. March 28th	April 20th	Quick
Myrobolan B (unworked)	..	..	..	.. February 20th	March 25th	Slow
Common Plum (unworked)	..	..	..	.. March 25th	April 5th	Quick
Hale's Early on St. Julien A	..	..	..	.. February 25th	April 7th	Very slow
Brompton (unworked)	..	..	..	.. April 1st	April 20th	Slow
St. Julien A (unworked)	..	..	..	.. April 1st	April 18th	Slow
St. Julien K (unworked)	..	..	..	.. March 28th	April 10th	Quick
Governor Wood on Mazzard	..	..	..	.. March 25th	May 4th	Very slow
Ukon on Mazzard	..	..	..	.. March 5th	April 20th	Very slow
Mazzard F5/1 (unworked)	..	..	..	.. March 20th	April 28th	Slow
Mahaleb F8/1/10 (unworked)	..	..	..	.. April 1st	April 18th	Quick

4. *Diploid and triploid pear grafts.*

Fortunately, the material available made it possible to ascertain whether any correlation existed between the chromosome make-up of stock and scion and incompatibility. In March 1936 Conference and Durondeau, two diploid pears (2n=34), were intergrafted and also reciprocally grafted with Pitmaston Duchess, a triploid variety (2n=51). The percentage take and the subsequent mean growth of the scions are given in Table XXIX.

TABLE XXIX.

Pear Grafting—Between Diploid and Triploid Varieties. All trees were worked on Quince A Stocks.

Combinations.			No. of grafts.	No. take.	% take.	First year shoot growth. cm.	S.E.
Pitmaston on Durondeau	..	..	30	20	67	131·5	15·9
Conference on Durondeau	..	..	30	30	100	118·8	7·5
Pitmaston on Conference	..	..	30	23	77	140·9	14·8
Durondeau on Conference	..	..	30	30	100	148·8	12·6
Durondeau on Pitmaston	..	..	30	29	97	111·2	15·9
Conference on Pitmaston	..	..	30	26	87	100·9	14·1
Durondeau on Durondeau	..	..	30	30	100	210·1	16·1

Although there are slight differences both in the percentage of take and in the subsequent average wood growth, yet they are not sufficiently significant to account for the degree of incompatibility.

Moreover, a chromosome study of Quince root tips has recently been made at the John Innes Horticultural Institution, showing that Quince A, C, F and G, which include two so-called compatible and two incompatible stocks, are all diploids, having a chromosome number $2n=34$. Therefore, the cause of incompatibility of Durondeau on Quince F, diploid on diploid, and the compatibility of Pitmaston Duchess on Quince A, triploid on diploid, evidently cannot be explained on the basis of the number of chromosomes.

5. *Annual growth curves of the unworked stocks and grafted scion varieties.*

The growth curves of the unworked and the grafted scion varieties were measured in the field. Stocks and one-year maiden trees (on compatible stocks) were planted in a uniform plot, having two replications in the row. Ten stocks of maiden trees were chosen at random for measurements during their second growing season. On each stock or tree, two terminal shoots were measured weekly in 1936 from bud-break until the cessation of growth. It was found that growth in length was fairly uniform within each set of ten trees.

The growth curves in Figs. 23-6 were prepared from these measurements. Each comparison of scion growth and stock growth on the graphs for grafted and ungrafted plants is the mean of measurements of twenty terminal shoots. Fig. 23 shows the weekly increments in shoot length for pears and Quince stocks and Figs. 24-6 show the total seasonal length for representative combinations in pears, plums and peaches.

It will be seen from Figs. 23-6 that the incompatible stocks had made not only much slower but also weaker growth. In addition, the growth curves of

PEARS AND QUINCE STOCKS
WEEKLY INCREMENTS IN SHOOT LENGTHS
1936

——— QUINCE A
•••• QUINCE F
—•—• PITMASTON
—○ ○— CONFERENCE
— — — DURONDEAU
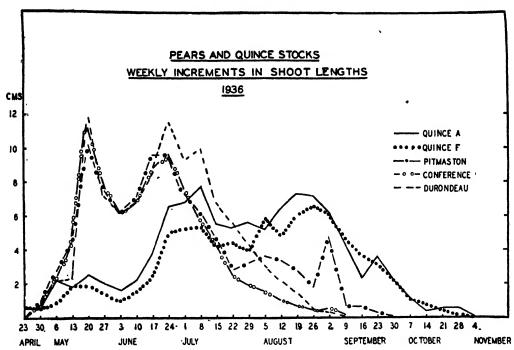
FIG. 23. Growth curves showing Weekly Increments in Shoot Lengths of Two Terminal Shoots. Pear and Quince Stocks.

SHOOT GROWTH OF PEARS
1936

DURONDEAU
PITMASTON
CONFERENCE
QUINCE A
QUINCE F
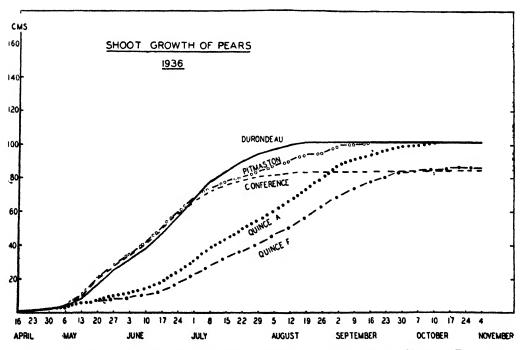
FIG. 24. Growth Curves showing Total Elongation of Two Terminal Shoots. Pear and Quince Stocks.

these incompatible stocks were quite different from those of the scions with which they proved incompatible.

In pears and quinces (Figs. 23-4) the curve for Quince F illustrates its slower and weaker growth as compared with that of Quince A and the three pear varieties. It is interesting to note that the growth of Conference, a variety which usually shows much delayed incompatibility, was complete very much earlier than that of Durondeau or Pitmaston Duchess.

Again, the growth curves of Victoria Plum and Common Plum stock (Fig. 25) are very similar, as also are those of Czar and Myrobolan B stock, two compatible combinations. But when the growth curves of Czar and Common Plum are compared very great divergence is apparent between the two, especially during the period May to July, when the trees were in their most actively growing condition. Although the growth curves of Victoria and Myrobolan B are also different, owing to the vigorous growth of Myrobolan B, they are much closer, as can be seen from Fig. 25.

The same phenomenon happened in peach and plum stocks (Fig. 26). Hale's Early Peach had a similar curve to that of St. Julien A, whereas it differed more from St. Julien K. The growth curves of Myrobolan B and Common Plum, two very incompatible stocks for Hale's Early Peach, differ strikingly from that of Hale's Early Peach.

When these growth curves were compared with the field observations, it was found that the incompatible combinations declined most rapidly during the period when the growth curves of the stock and the scion were at the most divergent stage. For instance, one-year-old Hale's Early Peach trees grafted on Myrobolan B, declined most rapidly at the end of July. The same phenomenon was observed on young grafts of Victoria Plum on Hale's Early Peach. At first, Victoria showed more vigorous growth than did Czar on peach stock, but about the middle of July, Victoria began to decline, and all the trees died at the beginning of September.

It appears (Figs. 23-6) that these differences in the rate and state of growth are of most importance during the main period of active growth. In all the incompatible combinations studied, the wide differences between their growth curves were most striking during May and up to the end of July. This is possibly due to the fact that during this period cell activity is at its height, and the wide differences in growth capacity at the union between stock and scion may then have a greater effect on the uniting tissues.

Finally, it was noted that when a weak growing variety was grafted on a strong growing stock, the grafts usually showed symptoms of incompatibility earlier and to a greater degree, than did those of a vigorous variety on a weak stock. This is shown clearly with Quince F on Durondeau, Common Plum on Czar, and Hale's Early Peach on Myrobolan B.
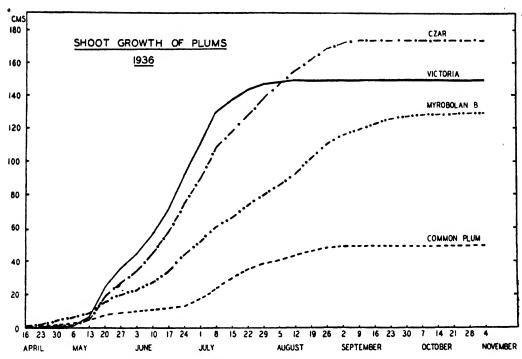
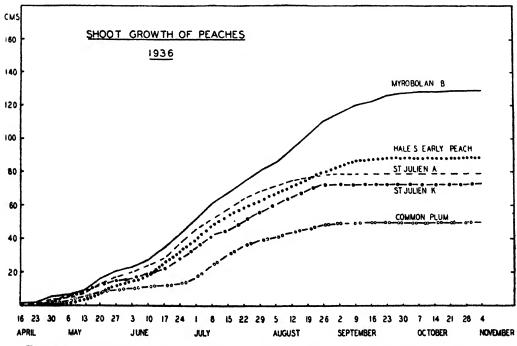FIG. 25. Growth Curves showing Total Elongation of Two Terminal Shoots. Plum and Plum Stocks.

FIG. 26. Growth Curves showing Total Elongation of Two Terminal Shoots. Peach and Plum Stocks.

V. DISCUSSION OF RESULTS.

The nature of compatibility and of incompatibility.

In Citrus budding, Mendel (39) observed the following processes of union in a normal and compatible combination :

1. First cell division   ..   ..   24 hours.
2. First callus bridges   ..   ..   5 days.
3. Cell differentiation (stock)   ..   ..   ..   10 days.
     ,,   ,,   (scion)   ..   ..   ..   15 days.
4. First occurrence of tracheids (stock)   ...   15 days.
     ,,   ,,   ,,   (scion)   ..   20 days.
5. First occurrence of meristematic layers in the
   callus   ..   ..   15 days.
6. Lignification of callus completed (stock)   ..   25-30 days.
     ,,   ,,   ,,   (scion)   ..   30-45 days.

Thus, in order to form a well-established growing tree by grafting or budding, there must be a series of processes to ensure the junction of the functioning cells at the union.   If the tree is to continue its normal development it should possess a healthy stem which can carry on the functions of an ordinary unworked tree trunk.   Any conditions which hinder, obstruct or destroy these vital cell formations at the union are detrimental to the growing shoots and the roots, and will result in one or more symptoms of incompatibility.

All plants are capable of regenerating organs or tissues when they are cut off from the parent, but this power of regeneration is limited in extent.   The uniting of stock with scion is accomplished partly by the regeneration of different sets of cells and partly by connecting together tissues performing similar functions belonging to the stock and the scion.   Proebsting (43) observed that in the formation of new tissues the differentiation of the cells of the scion appears to be influenced by the cells of the stock adjacent to the scion cambium.   That is to say, the cambium cells of the scion form medullary ray tissue when in contact with ray cells of the stock, and xylem tissue when in contact with xylem, etc. If these processes are all successfully completed the result will be a compatible union, resulting in a tree with a congenial and healthy stem.   On the other hand, should there be any great difference in the capacity of regeneration between stock and scion, there would be a corresponding variation in the period of cell differentiation, since the new meristematic cells regenerated from the stock would probably find no similarly active cells with which to unite in the scion. The result of this would be accumulation of dead cells and abnormal tissue formation at the union.   Such a combination of stock and scion is said to be incompatible.

The outstanding symptoms of incompatibility.

Growth decline.

Observations made in the course of the present study showed that incompatible combinations were sometimes associated with poor take of grafts and buds, especially in severe cases. They were always accompanied by subsequent decline in the area of foliage, in the amount of new wood, in new root growth and by other symptoms of ill-health, leading in most cases to the death of the tree.

Drying out of the stem and foliage, premature defoliation and premature flower bud formation seemed to indicate lack of water or of necessary nutrients in the scion.

In the experiments described, all the reciprocal (stock-scion and scion-stock) combinations revealed ultimately the same phenomena of incompatibility, but it must be noted that these results differ from those of Toxopeus (61) with *Citrus sinensis* and *C. Aurantium* grafts, and from those of Heppner and McCallum (24) with *Prunus salicina* and *P. domestica* grafts. These investigators did not get the same effects with reciprocal combinations.

Scion/stock ratio.

Vyvyan (63) observed that the total annual increment in weight of young trees was distributed in approximately constant proportions between roots, stems and leaves, irrespective of the size and exact age of the tree. Knight (31) showed that the ratio of stem increment to root increment is constant, within a given scion-stock combination, under East Malling conditions and as observed on winter pruned trees. He also showed that the scion/stock ratio is greater in larger than in small trees. The difference in the scion/stock ratio between compatible and incompatible combinations may therefore be due, at least in part, to the great difference in the size of the trees. When the scion proportion decreases, as shown in the decline of the annual wood growth in incompatible combinations, there would be a simultaneous decrease in the roots, and *vice versa*. But when the decrease on either side becomes too drastic, the other component cannot continue to function and death follows.

In the incompatible combinations, such as Durondeau Pear on Quince F or Hale's Early Peach on Myrobolan B, the scion portion was too small relative to the weight of the stock, as shown by the scion/stock ratio studies ; and, as was found in excavations, many of the old roots died back without being replaced to any appreciable extent. As the scion growth continues to decline, more roots will probably die back, until finally a limit is reached when the root system can no longer function. Thus, as the tree grows older, decline both in shoot and root growth becomes more marked.

The initially high scion/stock ratio observed in incompatible combinations may possibly be explained by the higher food reserve in the scion, due to obstruction of the downward movement of food materials at the union, while the water supply from the roots is still sufficient.

Maskell and Mason (38) have proved that organic nitrogen accumulates in the bark and wood above the ring in ringed cotton plants, and Clements (8) has shown that mineral materials move upward in the xylem in girdled pears and plums. It seems that the discontinuity in the bark and wood of incompatible combinations, described earlier in the present paper, acted at first like a ring or girdle around the stem. Actually, starch deposits were found above the union in the incompatible Durondeau on Quince F, as illustrated in Fig. 21, therefore there was an initially higher scion/stock ratio. When the roots were deprived of sufficient organic nitrogen and other elaborated plant food, they started to die back, and so, in turn, the shoot growth of the scion was reduced. Hence, the scion/stock ratio of the incompatible combination was subsequently much lower than that of the compatible one.

The immediate causes of decline in incompatible combinations.

It has been proved in the present study that all the incompatible combinations had mechanically weak unions, as shown by bark and wood discontinuity, the presence of parenchymatous cell accumulations, the relative ease of breaking and the smooth nature of the fracture at the union. These abnormalities had actually caused obstruction of water, soil nutrients and food transport, as was shown by the dye injections, water conductivity tests, and the accumulation of starch above the union.

The correlation between mechanical weakness and abnormal cell accumulation at the union with obstruction to the flow of water was suggestive. The experiment with negative pressure confirms Knight's (28) observation with positive pressure tests and proves that there was a big increase in resistance to the passage of water resulting from defective unions.

Abnormal cell enlargement and cell accumulations in incompatible unions were found on microscopical examination to be present within a few weeks after budding.

It is known that for grafts to be successful the scion must be kept as moist as possible to prevent drying out before actual union takes place. Therefore, the primary need of the scion is a sufficient water supply. In the early stage of union, however, the water requirement of the scion is small and sufficient water may be transported through the enlarged and irregularly arranged callus cells. But when the tree grows older and the scion becomes larger, the water transported through an obstructed union, together with a much restricted root development,

is insufficient to meet the increasing demands of the scion, especially during hot summer periods. Thus, the scion becomes dry, and finally dies back as a result of desiccation. This is most obvious in Common Plum on Czar, and Hale's Early Peach on Myrobolan B. The autumn defoliation of all incompatible combinations started from the tops of the branches instead of from their bases. The root system had apparently declined so much that there were not enough new roots to ensure a sufficient supply of water to the tops.

In peaches, excessive gum formation is probably an after-effect of the sudden change in the moisture content of the stem. This excessive gum formation inside of the conducting vessels will of course hasten the death of the scion. It seems to explain why Hale's Early Peach on Myrobolan B and Myrobolan B on Hale's Early Peach died back more rapidly than incompatible combinations of pears and plums, where gum formation was absent.

The possible fundamental causes of incompatibility.

Previous workers (15, 46, 14, 16) have shown that between plants of distantly related groups there are significant differences in nutritional capacities and in enzymatic or precipitin reaction. This bio-chemical aspect of the problem has not been included in the present investigation which is concerned only with plants of related groups.

On the physiological side, it has always been difficult to explain why the same variety should be compatible with one selection of stock and incompatible with another. Field observations showed indications of the existence of wide differences in certain physiological functional capacities between incompatible and compatible stocks and scions, as has already been discussed. The author therefore devoted more attention to some of these physiological functional differences in the deciduous fruit trees investigated. Incidentally, some interesting results have been obtained as described in the previous sections.

Period of cambial activity.

The present investigations showed that there were differences in the periods of cambial activity between the scion and its corresponding incompatible stock, as shown by the peeling of the bark and by strippings, using the method described by Priestley (42).

Knight (29, 30) and Swarbrick (59) observed on apple and plum trees, that cambial activity starts in the region of buds that are developing into new shoots, and spreads downwards from these through the stem. Moreover, Haley (18) found that there was more rapid or slower spread of cambial activity and vascular differentiation in the stems of different forest trees such as Quercus and Aesculus.

With these results in mind, it is not surprising to find that when a scion variety of late or slow cambial activity is grafted on a stock variety of early or rapid cambial activity, constant breaking and " re-grafting " of the uniting cells takes place and that a large accumulation of inert functionless tissue occurs at the union, such as has been found in incompatible combinations.

However, contrary to the statement made by Swarbrick (59), to the effect that the cessation of cambial activity works basipetally in the stem of an ordinary healthy tree, the results of the present experiments would appear to show that in incompatible combinations, such as Czar on Common Plum, or Durondeau Pear on Quince F, the cessation of cambial activity in the stock starts from the union and proceeds downwards, somewhat independently of the cambial activity in the scion.   In these cases (Table XXVII), the stocks showed a stiffer bark and thinner strippings much earlier than did the stem portion of the scion just above the union.

The duration of leaf-expansion after bud-break, as observed in the present investigation, may be associated with cambial activity.   The shorter period between bud-break and leaf-expansion observed in Common Plum and Mahaleb seems to indicate more rapid cambial activity in the tree (Table XXVIII).

Period of callus growth.

The callus studies of grafts, already described, also revealed functional differences in the efficiency of callusing between different stocks and scion varieties.   Sharples and Gunnery (56) stated that the cambium plays no part in the early development of callus, which is predominantly the product of medullary ray cells.   It seems certain, however, that all the water supply of the scion has to be transmitted from the stock through this " transitory callus bridging ".   Callusing is in fact the necessary first step for the formation of permanent conducting tracheids, as Mendel (39) observed.   If either the scion or the stock were delayed in its period of callus bridging, there would also be a delayed period of tracheid strand differentiation.   In the absence of corresponding connecting tissues, the earlier growing callus and tracheid strands would dry out and become dead cells, as shown in Fig. 22.   When the callus tissues from the delayed scion or stock begin to grow, they find no corresponding meristematic cells with which to unite.   Sorauer (58) pointed out that in setting grafts, actual contact of cambium layers is not essential, and that it is in practice rarely achieved, but contact of cambium-derived callus is important.   Thus, if the variation in time in the regeneration of callus tissue between stock and scion is very great, the scion will not grow or will make a very poor start and then dry out.   But if the variation is less pronounced there will be constant breaking and re-grafting of the tissues which are uniting with each other.   Thus, a thick layer

of enlarged cells or cell accumulations will be formed, or there will be inwardly curved layers of cells as manifested in the clean breaking-out of incompatible combinations (Fig. 18). Both scion and stock will be weakened by this obstruction at the union.

Growth curves.

When the growth curves, representing the extension growth of two terminal shoots, were compared it was observed that there were differences not only in the times of starting and cessation of growth but also in the periods of most rapid growth. As already discussed, the combinations were generally incompatible when the growth curves of the stock and scion were most unlike. The best example can be seen in Czar on Common Plum. It may further be proved in Victoria and Czar plums grafted on Hale's Early Peach. Czar showed a similar growth curve, and Victoria a very different one, compared with Hale's Early Peach. Eventually, all Victoria on Hale's Early Peach were defoliating and dead by the end of the first season. The decline in growth in the incompatible combinations was usually most rapid during the period when the growth curves showed most divergence.

Moreover, incompatibility appeared earliest and in its most acute form in those combinations in which the growth curve of the scion was weaker than that of the stock. This may be due to the normal variation in growth activity between a scion and a well-established rootstock. In the reciprocal combinations in which the growth curve of the stock was the weaker, the symptoms of incompatibility are delayed.

The fact that the degree of incompatibility was more severe in older trees may be explained by the breaking down of the tissues at the union in successive seasons. As the tree grows older and taller, these growth activities starting at the growing tips have to travel a much longer stem before they can reach the union. Thus, the variation in the time of commencing and cessation of these growth activities between the cells of the scion and stock at the union will be greater. Swarbrick (59) noticed that in apple branches, the initiation of xylem formation began in the apical regions and worked downwards, there being at least three weeks' difference between its initiation in the one- and the four-year-old branches.

It is reasonable to assume, therefore, that these differences in cambial activity, in capacity for callus tissue generation, in growth curves and in the rate of transmission of these activities through the stem form an important part of the causes of incompatibility.

Indeed, it is a common practice to store scion wood before grafting in order to get delayed dormancy and thus to synchronize the cambial activity and callus

growth in scion and stock.   This result is also sometimes attained by treating the scion or the stock with certain chemicals so as to stimulate the growth activities of the cells at the union.   Gossard (13) found that he could get a higher percentage of graft take by treating the scion wood with chemicals.   But since all these physiological functional capacities are inherent, the successful uniting together of the conducting cells by artificial inhibition or stimulation is only temporary and will break down again in the following season.

All these physiological growth activities are extremely susceptible to the influences of soil and climatic conditions, as is observable in the field.   Therefore, it is not surprising to find contrasting cases of compatibility and incompatibility with the same given scion and stock in combination.   Work done at East Malling (1) has shown the variable results obtained in the nursery from year to year.   Swingle (60) and Shippy (57) showed that the callusing of apple varieties differed in regard to optimum temperature requirement, as well as in the duration of maximum activity and the dates of beginning and end.   Again, Howard and Heppner (27) showed that some strains of Myrobolan Plum were generally successful as peach stocks in California.   But when these Californian strains of Myrobolan were sent to East Malling and were worked with Hale's Early Peach scions, none of them showed any sign of compatibility with this variety.

Moreover, since all these growth activities are inherent, they may be transmitted and segregated in the progenies after crossing two parent varieties. Crane has informed the present author that he observed on crossing Durondeau with Dr. Jules Guyot (a pear variety which usually fails on nearly all quince varieties), that there were a few seedlings in the F_1 generation which were incompatible with Quince C.

From the practical point of view, slight incompatibility may render a tree less vegetative in its growth and more prolific in bearing.   As with pears on Quince A and Quince C, there was noticeable discontinuity in the outer cortical region whilst both the bark and wood fibres were continuous (see Fig. 13).   Such slight discontinuity may have a similar effect to ringing or girdling, as sometimes practised on extremely vigorously growing trees.   Kraybill (36) has proved that ringing lowers the moisture and total nitrogen content above the ring, but increases the reducing sugars, thus resulting in a higher carbohydrate/nitrogen ratio. Again, du Sablon (54) has shown that there is a higher starch content in Duchesse d'Angoulême pear scion in all seasons, when grafted on quince than on pear stocks.

Thus, it would even appear that the use of a rootstock which is not entirely compatible may sometimes be preferable, from the growers' point of view, to that of a stock which shows no incompatibility.   But it would be highly detrimental to grow trees on very incompatible stocks, a proceeding which may result in quick decline and premature death in the nursery and orchard.

SUMMARY.

1. The problem of incompatibility between stock and scion has been studied in deciduous fruit trees, especially in pears, plums, peaches and cherries. Some 86 grafted and 32 budded combinations of scion-stock, stock-scion and stock-stock have been studied at East Malling during 1935-37. All the rootstocks used were of clonal origin and were vegetatively propagated.

2. With all incompatible combinations, grafted or budded, there were marked symptoms of a low percentage take, premature autumn leaf-coloration and flower bud formation, early defoliation, and dying back of young shoots. The low percentage take was less noticeable on budded than on grafted plants. Defoliation started from the tops of the shoots in incompatible combinations in contrast to the ordinary defoliation from the basal portion of the shoots in compatible combinations.

3. In all incompatible combinations, there was a marked gradual decline of the annual wood growth, usually following an initially vigorous growth at an earlier stage. There was also a gradual decline of new root growth both in number and length. The incompatible combinations started new root growth much later in the spring than the compatible ones. These findings corresponded with the results obtained from excavated trees, namely that the incompatible combinations had an initially higher but subsequently much lower scion/stock ratio than the compatible ones. All these symptoms were intensified in direct relation to the age of the tree and the degree of incompatibility. Moreover, these symptoms occurred both in scion-stock and their reciprocal combinations.

4. All incompatible combinations showed a mechanically weaker union than the compatible ones. This was indicated by :

(a) Bark and wood-fibre discontinuity. Incompatible combinations showed three classes of bark and wood-fibre discontinuity, arranged in order of decreasing severity, as follows :

   (i) Both bark and wood fibres discontinuous.

   (ii) Bark continuous, but wood fibres discontinuous.

   (iii) Bark discontinuous, but wood fibres continuous.

(b) Heavy accumulation of parenchymatous cells at the union. Compatible combinations possessed regular strands of medullary ray cells across the union about four weeks after budding ; whereas the incompatible ones had much enlarged parenchymatous cells arranged irregularly at the union even up to twelve weeks after budding.

(c) Reduced strength at the union and clean surface of break. Incompatible combinations required far less force to fracture the union than compatible ones,

and the surface of the fracture was invariably smooth and had a distinctive layer of inwardly curved cells.

5.   Incompatible combinations had some form of obstruction at the union. This was indicated by :

(*a*) A slower flow of dyes through the union, as shown by dye injections.

(*b*) A much higher resistance to the flow of water, as shown by water conductivity tests.

(*c*) A heavy deposit of starch above the union in November, as shown by the iodine test.

Mechanical weakness and obstruction at the union are suggested as the immediate causes of the decline of a tree showing an incompatible combination.

6.   Some of the physiological functional differences between the scion and its incompatible stock have been studied.   It was found that different stock and scion varieties had :

(*a*) Different periods of cambial activity, as observed by the bark-peeling and stripping method, in summer and autumn.

(*b*) Different periods of callus growth and different regions of callus differentiation, as studied in grafts.   In incompatible combinations, most of the callus cells which were not in contact died after about a month.

(*c*) Difference in the duration of leaf-expansion from the time of bud-break. The combination was most incompatible when a slow leaf-expanding scion was worked on a quick leaf-expanding stock.

(*d*) Difference in growth curves, as shown by the weekly measurements of the terminal shoots.

The wider the divergencies of such physiological functional differences between a scion and its stock, the greater the degree of incompatibility.

7.   It is concluded that a mechanically weak union and the increasing obstruction at the union are the immediate causes of shoot and root decline in an incompatible combination, and that all the physiological functional differences manifested in cambial activity, callus growth, duration of leaf-expansion, and growth curves of the stock and scion varieties may be accounted as important contributory causes of incompatibility among deciduous fruit trees.

8.   It is pointed out that all these physiological functional differences in the growth activities of the stock and scion are inherent, and may be somewhat modified under different climatic and environmental conditions, and this helps to explain the different results obtained in different parts of the world in regard to the occurrence of the symptoms of incompatibility.

ACKNOWLEDGMENTS.

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A STUDY OF THE TECHNIQUE OF VARIETY TRIALS, AS ILLUSTRATED BY THE COMPARATIVE YIELDS OF FOUR BLACK CURRANT VARIETIES GROWN IN THREE DIFFERENT LOCALITIES

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THE adequate testing of new varieties of commonly grown fruits is a difficulty with which modern horticultural research has to contend, and in recent years it has become more and more exacting. The flow of new varieties is unceasing ; and for the protection of the grower it is essential that their qualities should be carefully tested by an impartial authority. While the final decision as to whether or no he adopts a new introduction must rest with the grower himself, much can be done to assist him by passing the flow through a series of sieves in order to eliminate all but a few of the most worthy.

Such a grading system has been provided by the National Fruit Trials which, for the past sixteen years, has been employed by the Royal Horticultural Society under the aegis of the Ministry of Agriculture.

The varieties are sent in to the Society's gardens at Wisley and are there kept under close observation for a period of years in comparison with standard varieties of proved quality and yielding capacity. The plan of this preliminary testing need be only of a simple nature, for the differences to be looked for are chiefly in the quality of the fruits and on a large scale ; and unless a new introduction shows some marked improvement on existing sorts, it need be considered no further. It is necessary only to have sufficient plants for each variety to be fairly represented, and to arrange them in such a way that the sources of error to which they are subject are comparable.

From this first test a few varieties may emerge good enough to undergo a much more severe trial. Are they in fact worthy of introduction in commerce ?

While certain varieties may be excellent for garden use, the demands of the market grower are often very different. Mere excellence of flavour is for him not sufficient—indeed there are certain attributes which to him are equally, if not more important, and the chief of these is yield. Further, while a variety may give good returns under the sheltered conditions of a private garden, how will it survive an altogether harder existence in the open field ? And will it "do" in his field ? In other words, is the variety of general value or useful only under particular conditions of soil and climate ?

These questions are very much more difficult to answer, involving, as they do automatically, a series of trials in as many places as possible. Further, the performance of varieties in commercial use to-day is of a high order, and increases in yield are likely to be comparatively small. Not that these increments may not be of commercial value, but a much more rigorous test is necessary to establish or disprove their existence.

It was in order to find a suitable method for this kind of trial that the present series of experiments was initiated in 1927 by the Joint Committee who administer the scheme, in collaboration with the research institutions concerned.

MATERIAL.

For the time being the investigation was confined to small fruits, and the black currant was adopted as a suitable indicator crop.

Only three stations collaborated in the work, since, in order to find the best method, the trials planned were much more elaborate than was then deemed necessary, and these stations alone had the facilities for recording and the land available. The three research institutions concerned were the Royal Horticultural Society at Wisley, Surrey, the Long Ashton Research Station, Bristol, and the East Malling Research Station, Kent.

The soil at Wisley is a sandy loam with a similar but slightly heavier subsoil, and the station has an annual rainfall of about 25 inches. Long Ashton is situated on a fine sandy loam overlying a marly subsoil, and has an annual rainfall of 34 inches ; while at East Malling the soil is a good medium loam over the Kentish ragstone, and the rainfall averages about 25 inches.

The varieties chosen were two standards, well known in commerce, Boskoop Giant and Baldwin, and two new introductions which had already passed a preliminary test at Wisley, Davison's Eight and Taylor's 21/23. The two standards were selected to represent early and late bearing varieties. While they are typical of the varietal groups to which they belong, they do not cover the whole range of black currant varieties, representatives of the " French " and " Goliath " groups being absent. There was thus no strict comparison, for instance, between Davison's Eight, which is nearest to the Goliath group though of the same season as Boskoop, and a standard variety of the same type. One hundred bushes of each variety were distributed to each of the three participating stations, all the material having been propagated at Wisley.

THE EXPERIMENTAL DESIGN.

In 1927 information as to best size of plot and number of replications was not very extensive. Hatton and his co-workers had recently published their paper (1) on Black Currant Variety Trials and had stated that fifty-seven bushes of each variety would be required to prove a mean difference of 10% to be

statistically significant, these figures being based on the totals of three cropping seasons.

However, the bushes in that trial were not so arranged that the effect of soil differences could be eliminated ; and there was no information as to the accuracy which might be achieved by the use of the then fairly new methods of plot arrangement, such as the Latin square, recently developed by Fisher at Rothamsted.   Accordingly, in the present experiment, it was decided to allow ample numbers, so as to hit the optimum as nearly as possible and to try how far a randomized pattern could be adapted to the needs of the trial.

The next point to be decided was the experimental unit.   In experiments with trees in England it is usual to adopt the individual tree as the equivalent of an agricultural plot.   However, to distribute one hundred individual bushes of each of four varieties at random would have been an obvious absurdity, and a plot of four bushes was adopted as the smallest unit which could conveniently be harvested and recorded.

The four varieties were then arranged in a series of six four by four Latin squares, each square containing four plots of four bushes of each variety. There were thus ninety-six bushes to be recorded in twenty-four plots of four bushes for each of the four kinds.   It is hardly necessary to explain the nature of the Latin square design now, but it may be stated briefly that in it there are the same number of plots of each variety as there are varieties, and that each variety occurs once in each row and once in each column of a square pattern. It is also part of the definition that each new square used should be picked at random from all possible squares which fulfil the first two conditions.

In the present experiment the six such squares were to be laid down adjacent to one another at each station.   This was carried out at Wisley and at East Malling, the only difference between the two being that at the latter station the individual rows of black currants were interplanted with raspberries.   This may have increased the plot to plot variability, owing to varying competition with the intercrop.   At Long Ashton it was not possible to carry out the scheme exactly ; owing to the shape of the ground only four squares were possible, and two sets of four randomized blocks.   Further, the four squares used were identical in pattern.

The three trials were planted in the winter of 1927-28, and annual records of yield were taken until the summer of 1933.   Omitting the few fruits in 1928, the following discussion is based therefore on the yields of four-bush plots in five consecutive seasons.

THE RESULTS.

(i) *Individual Trials.* The results were examined by the analysis of variance method (2), and the following Table shows the average yield over the

whole period in kilograms per plot of the four varieties at each station, together with the standard error appropriate to the averages in each trial, and the difference necessary to give a 20 to 1 chance in favour of significance. In order to make a comparison of the three trials, these varietal averages are also given as percentages of the mean yield of all four varieties in each.

TABLE I.

Total crop for five-year period in kilograms per plot of four bushes and as a percentage of the general mean of all four varieties at each station.

		Boskoop.	Baldwin.	Davison's.	Taylor's	Gen. Mean.	S.E.	Sig. Diff.
Wisley	Kilos	26·7	21·8	15·6	25·5	22·4	0·48	1·4
	%	119	97	70	114	100	2·1	6·2
Malling	Kilos	21·5	31·3	18·3	2·0	18·3	0·48	1·4
	%	117	171	100	11	100	2·6	7·6
Long Ashton	Kilos	34·7	24·8	14·3	12·2	21·5	0·61	1·7
	%	161	115	67	57	100	2·8	8·1

The chief point of interest in this Table is the accuracy of the results. The last two columns show the standard errors and the differences between means necessary for significance. These last vary from about 6% at Wisley to 8% at Long Ashton. As might be expected from the large number of replications and accurate technique, the differences which the experiment is capable of establishing are fairly small compared with those obtained in many horticultural trials. That at Wisley gives the best result, probably because only here was the full benefit of the Latin square design felt. At East Malling, probably owing to variable interplanted raspberries, the variations between like plots were not markedly connected with definite positions in the field. Thus the effect of position could not be eliminated in the statistical analysis to any great extent. At Long Ashton, again, a somewhat less accurate layout was used, so that both these stations gave a rather lower degree of precision than Wisley.

Still, regarding these as individual trials, it is possible to say that at each one of these stations the method employed was capable of giving a comparison of the four varieties, over the period concerned, of very fair precision. In actual fact very large differences in yield were observed at all three stations, but even if they had been of a much smaller order, a definite ruling could have been obtained on any difference of commercial importance.

But there is much more to be learnt from these results than this. Up to this point they have been considered as the results of three unconnected

trials. If this had really been the case, it would not have been possible to argue further, for any difference in the response of the varieties to particular conditions at these three stations might have been attributed to the material, the age of the bushes or a difference in planning. But in the present example these three factors are practically identical, except for small deviations in the last, which have already been shown to have decreased only slightly the accuracy at the stations concerned. It is therefore perfectly justifiable to analyse the three trials as a whole. This will yield very much more information ; for now it is possible to evaluate accurately the response of the four varieties to varying conditions of soil and climate. Table II (a) tabulates the mean yields for the five-year period, this time as a percentage of the mean yield of all stations and all varieties.

TABLE II (a).

Average yield of four varieties for all stations, as percentage of the mean of all four.

Boskoop.	Baldwin.	Davison's.	Taylor's.	Gen. Mean.	S.E.	Sig. Diff.
134	125	77	64	100	1·5	4·2

In this Table the yields are those of the seventy-two plots of each variety at the three stations. It may be asked, what is the value of an average yield from these so widely different places ? If a new variety is to be of commercial value it must succeed in a majority of those districts in which black currants are likely to be grown. If such is the case, it should yield a higher average, taking into account all the places tried, than others in the trial. The above Table gives an accurate comparison of the merits of the four varieties for general purposes, in so far as the yields of three stations can give that information. Evidently the greater the number of stations, the greater the value of these grouped yields.

Naturally, since there are three times as many plots, the accuracy is greater than that obtained at a single station, the difference necessary for a 20 to 1 chance being reduced to 4·2. It is evident that on the whole the two varieties have not stood the test of comparison with the two established varieties Boskoop and Baldwin, and cannot therefore be recommended for general use. Of the two former, Boskoop has been the heavier cropper, while Davison's has yielded more than Taylor's. But, again, this is by no means the whole story, as is shown in Table II (b).

Although unsuitable for general introduction the two new varieties may have a value for particular soils or climates. The analysis in this case showed clearly that although the grouped yields showed definite differences, the relative yields of the four varieties were not alike for all stations.

In this Table the relative mean yield of each is that of twenty-four plots, as in Table I, and the significant difference, as might be expected, is intermediate between those of the three stations.

TABLE II (b).

Average yield of the four varieties at each station.

	Boskoop.	Baldwin.	Davison's.	Taylor's.	Gen. Mean.	S.E.	Sig Diff.
Wisley	129	105	75	123			
East Malling	104	151	88	10	100	2·6	7·3
Long Ashton	167	120	69	59			

It is quite clear that the varieties have responded differently to the conditions in these localities. Boskoop and Baldwin have both been good in all three places, though not always in the same order. The former has cropped the better at Wisley and Long Ashton, while the latter was considerably the heavier cropper at East Malling. Of Davison's it can be said only that its performance has been generally mediocre, although it may still be an improvement on other varieties of the same varietal group. But in comparison with Boskoop and Baldwin, unless it has some outstanding quality other than yield, it is hardly worth introduction. Taylor's, on the other hand, appears to be much more particular, being outstanding at Wisley, poor at Long Ashton, and a complete failure at East Malling.

The reasons for these varietal preferences are more difficult to define, and, as they are not material to the present study, will not be considered here. However, a consideration of the annual crops, depicted graphically in Fig. 1, shows that these results are fairly consistent from year to year, the relative cropping of the varieties being roughly the same both in good and bad seasons. The only marked departure is the comparative behaviour of Boskoop and Baldwin in different years at East Malling, and the marked improvement of Davison's as compared with Taylor's at Long Ashton in the last two seasons.
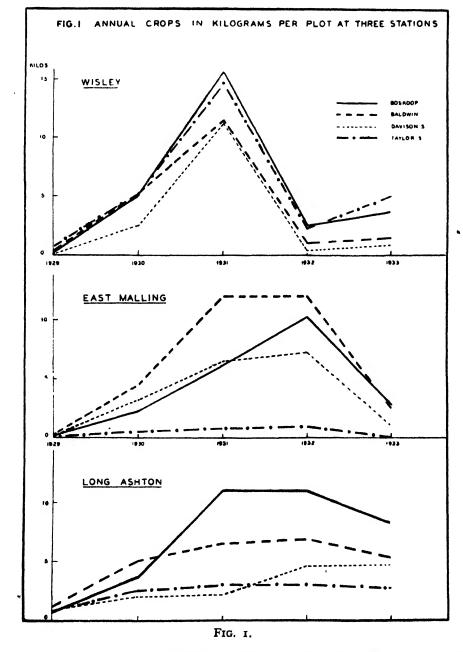
It may be considered therefore that, in so far as the five seasons during which the experiment was in progress are concerned, the relative cropping value of these varieties for the three stations under the conditions under which they were grown, is definitely established.

How far are these results of general applicability, and if they are not, does the experiment give any guidance for such trials in the future ?

Evidently this three-fold trial has given results of much wider application than merely a single trial could have done, but it is obvious that, as far as the fruit growing areas of England are concerned, a wider range of soils and

climates would add very greatly to the confidence with which the findings could be regarded.

To carry out such trials as these, however, would be beyond the powers of any but fully equipped and staffed experimental stations.   Nor are such accurate

FIG. I. ANNUAL CROPS IN KILOGRAMS PER PLOT AT THREE STATIONS

FIG. I.

trials necessary.   A series sufficiently precise to prove definitely the occurrence of increases in crop of 10% over a wide range of stations or 15% at a single station would probably be ample for present purposes ; since, if there is no

greater difference than this, qualities other than yield are likely to be the deciding factor as to a variety's adoption or rejection.

The present data will therefore next be used to indicate the accuracy which might be attained with varying sizes of experiment at a single station, and the improvement to be obtained by increasing the number of stations.

For the present a single four by four Latin square is taken as the smallest arrangement for comparing four varieties, and the effect of increasing this from one to six squares is shown. It may be noted here that the four-bush plot is not necessarily the most economic unit and, where land is a smaller consideration than labour, it might be better to increase the plot size rather than the number of replications. However, the present experiment does not readily lend itself to a discussion of size of plot, and only the four-bush plot will be considered here.

EFFECT OF INCREASING REPLICATION ON PRECISION.

The precision of an experiment depends upon three factors, the variability of the material, the number of replicated plots, and the method of arrangement. In this case the last factor is constant, i.e. the four by four Latin square, so that for present purposes it is necessary to consider only the first two.

In planning an experiment two points must be considered. There must first be sufficient repetitions of like plots to give a reasonably good estimate of the variability of the material, and secondly there must also be enough to give a reliable estimate of the mean yields of the varieties in the trial. If the first condition can be predicted in advance, it is possible to estimate very closely how many repetitions are necessary to give any desired degree of accuracy in the estimates of mean yield.

The value usually employed to give an estimate of the variability of the material is the coefficient of variability (V). This is simply the standard deviation (σ) as a percentage of the general mean of all plots. Having obtained an estimate of the standard deviation, the errors to which any particular mean is subject are calculated by dividing by the square root of the number of repetitions of which it is composed. This is known as the standard error of the mean. In considering the difference between any pair of means in the experiment the standard error of the difference must be found. This is the standard deviation divided by the square root of half the number of repetitions, where this number is the same for each variety. In order to estimate the significance of any particular difference, it is divided by its standard error to obtain a figure known as t. Tables are available (2) in which can be found the value of t necessary to give a 20 to 1 chance in favour of significance. t varies according to the size of the experiment. Where it is on a large scale the value is about two, but if the experiment is more limited t must be rather higher to give the same level of significance.

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Thus, if V is known it is a simple matter to calculate not only the expected standard error of the mean yields, but also the percentage difference, the significance of which any particular experimental design may be expected to prove, i.e.

$$\text{Standard Error (per cent.)} = \frac{V}{\sqrt{n}}$$

$$\text{Significant Difference (per cent.) } (P = \cdot 05) = \frac{V}{\sqrt{\frac{1}{2}n}} \times t.$$

Where n is the number of replications.

(Note, a probability $(P = \cdot 05)$, gives odds of 20 to 1 in favour of significance.)

In the following Table III the percentage standard errors appropriate to varietal means, which would be expected at two individual stations, are tabulated for designs embodying from one to six four by four squares. The best estimate of the coefficient of variability at each station is that obtained when the yields of all six squares are analysed together, and this is used for the calculation.

TABLE III.

Comparison of the precision expected and obtained by increasing numbers of four by four Latin squares at two individual stations.

	Number of Squares.					
	1	2	3	4	5	6
WISLEY						
(No. of Plots)	(4)	(8)	(12)	(16)	(20)	(24)
Standard Error %						
Expected (V = 10·6)	5·3	3·7	3·1	2·7	2·4	2·1
Obtained	5·7	3·1	3·3	2·7	2·4	2·1
Significant Difference % (P = ·05)	19·5	9·4	9·5	7·9	7·0	6·2
EAST MALLING						
(No. of Plots)	(4)	(8)	(12)	(16)	(20)	(24)
Standard Error %						
Expected (V = 13·0)	6·5	4·6	3·8	3·2	2·9	2·6
Obtained	6·2	4·7	3·9	3·6	3·1	2·6
Significant Difference % (P = ·05)	21·7	14·3	11·3	10·3	8·9	7·7

With these are compared the actual standard errors obtained by taking at random, for analysis, first one square, then two squares, and so on until all six have been used from each station. From these latter are calculated the actual significant differences which each of the six combinations was able to prove. Thus, in a single experiment at one station, it might be expected to prove differences between means of about 20% if a single four by four square were used. If the size of the experiment were doubled, this would be decreased to about 13%, and if trebled, to about 10%. Subsequent additions do not greatly

increase the accuracy, the significant difference decreasing about 1% for each new square added. It is interesting to note that on the whole the standard errors obtained agree very closely with those expected. The larger deviations occur where the number of squares is small. This may well be expected, for with so few replications the estimate of the coefficient of variability would not be very reliable.

It is of interest to compare these results with Hatton and his co-workers' fifty-seven bushes to show a 10% difference, mentioned earlier. This estimate apparently agrees fairly closely with the new results at East Malling. (Between three and four squares, i.e. about fifty-six bushes.) Where, however, the Latin square design was able to work more efficiently at Wisley, three squares, i.e. forty-eight bushes per variety, would have been ample to give the same precision.

TABLE IV.

Comparison of precision expected and obtained by increasing numbers of squares when two stations are analysed together.

Number of Squares Number of Replications	1 (4)	2 (8)	3 (12)	4 (16)	5 (20)	6 (24)
AT EACH STATION Standard Error %						
Expected ($V = 11 \cdot 7$)	**5·8**	**4·1**	**3·4**	**2·9**	**2·6**	**2·4**
Obtained	5·9	3·8	3·5	3·1	2·7	2·4
Significant Difference (P = ·05)	18·3	11·1	10·1	8·8	7·7	6·8
AT BOTH STATIONS Standard Error %	(8)	(16)	(24)	(32)	(40)	(48)
Expected ($V = 11 \cdot 7$)	**4·1**	**2·9**	**2·4**	**2·1**	**1·8**	**1·7**
Obtained	4·2	2·7	2·5	2·2	1·9	1·7
Significant Difference (P = ·05)	12·9	7·8	7·2	6·2	5·4	4·8

In Table IV the results from both stations are analysed together, and the resulting standard errors obtained compared with those expected. As will be seen, the agreement with expectation is closer here, as now even where there is only one square at each station the coefficient of variability is calculated from quite a considerable number of plots.

If the varietal means at each station are compared, the accuracy is intermediate between that obtained where the two single stations are analysed separately ; but if the varietal means for both stations together are compared, a considerable increase in accuracy is obtained. Thus, with one square at each of two stations, a difference of about 13% in average yield would be significant ; with two squares, about 8%, with a further reduction to 5% where all six squares are used.

In Table V the data from all three stations are analysed together from one to four squares. In this case the coefficient of variability on which the expectations are based is rather higher, since plots at both Long Ashton and East Malling were more variable than at Wisley.

TABLE V.

Comparison of precision expected and obtained by increasing numbers of squares when three stations are analysed together.

Number of Squares	1	2	3	4
Number of Replications	(4)	(8)	(12)	(16)
AT EACH STATION				
Standard Error %				
Expected (V=12·6)	6·3	4·5	3·6	3·2
Obtained	7·8	3·9	3·8	3·2
Significant Difference (P=·05)	23·2	11·1	10·7	8·9
AT ALL THREE STATIONS	(12)	(24)	(36)	(48)
Standard Error %				
Expected (V=12·6)	3·6	2·6	2·1	1·8
Obtained	4·5	2·2	2·2	1·8
Significant Difference (P=·05)	13·5	6·4	6·2	5·1

There is a fairly wide deviation between the standard errors expected and obtained for one square, illustrating the comparatively low reliability of the coefficient of variability where numbers are small. For the rest, the significant differences between means at individual stations are about the same as in Table IV, but there is a further increase in precision when the means of the four varieties at all three stations together are compared. Two squares at three stations will prove a difference of about 7% and four squares will give about the same precision as six squares at two stations.

To sum up, it seems likely that from three to four four by four Latin squares would be ample for a single experiment in one place to show differences of the order of 10%. If the experiment were repeated at two or three stations, two squares, i.e. eight replicated four-bush plots, would be enough to prove definitely the existence of differences in the mean performance at all stations of 8% ; while if the varieties do not respond alike to the varying soils and climates in different places, differences at individual stations of 13% would be significant.

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SUMMARY.

The yield data of four varieties of black currant over a five-year period
secured from an extensive experiment carried out simultaneously at three
stations are examined.

It is shown that the experimental design used, namely six four by four
Latin squares with a four-bush plot as the experimental unit, gave exceptionally
accurate results.   Large differences were shown not only between the gross
varietal yields at all stations, but also in the response at individual stations.

These data were used to determine the best number of replicated plots for
use in such trials.   It is concluded that whereas from three to four four by four
Latin squares would be necessary for reasonable precision at a single station,
two similar squares would be ample if the experiment were repeated at two or
three stations.

LABORATORY METHODS FOR THE BIOLOGICAL TESTING OF INSECTICIDES

I. METHODS OF TESTING OVICIDES

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INTRODUCTION.

THE number of materials requiring consideration as ingredients of horticultural sprays has vastly increased in recent years.   From such a host of potential insecticides the few worthy of close investigation can be sorted out only by rapid and reliable laboratory methods of testing.   Accurate biological tests are also called for in any attempt to correlate chemical constitution with insecticidal action and thereby rationalize the search for new insecticides and new combinations of spray materials.

Laboratory tests of a fairly rough type have long been employed at East Malling to gain preliminary information concerning the behaviour of insecticides intended for field trial, but the need for more refined methods led, in 1933, to the start of a special study of the whole problem.*   The aim throughout has been to devise simple methods that will give reproducible results in tests of ovicides, contact insecticides and stomach poisons.   Two main considerations have influenced the development of these methods.   These are, the necessity for standardizing both the biological material and every stage in procedure, and the desirability of using as test insects only such species as are representative of the several types commonly occurring as pests of fruit trees and bushes.

Experience gained in this investigation has shown that the biological testing of insecticides is best carried out in four stages.   These are :

A. Preliminary, successive " sorting tests " carried out in the laboratory.

B. Investigation of the phytocidal properties of the more promising materials selected in stage A.

C. Small field trials, followed by larger ones, of the more promising of the substances that stage B has shown can safely be applied to fruit trees and bushes.

D. Field trials of selected substances as components of the routine spray programme.

The scope of the present paper is confined to stages A and B in so far as they concern ovicides.

* The following have at various times taken part in these investigations: Messrs. G. L. Hey, F. J. D. Thomas and G. A. Brett.   To them, and particularly to Mr. Thomas, credit is due for devising many details of the methods that have been developed.

THE CHOICE OF TEST INSECTS.

Species selected for regular use in laboratory trials should satisfy as many as possible of the following requirements:

1. *Ease of rearing.* They should pair readily in captivity and lay eggs in conveniently arranged groups and in accessible positions. They should show no tendency to cannibalism and they should be hardy and thus able to withstand frequent handling.

2. *Ease of handling.* Some insects can be picked up with a brush more readily than others and can thus be more rapidly transferred to clean cages and fresh food. External feeders are much easier to handle than insects that feed in the interior of stems or that spin leaves together and feed between them. Again, some crawl rapidly and are difficult to retain in the desired position for spraying. For tests of ovicides it is essential to use insects that will lay eggs on muslin, twigs or other material that can readily be treated with an insecticide, and in such a manner that they can be counted accurately and easily.

3. *Ability to make use of a wide range of host plants.* Insects that feed equally well on several different host plants are to be preferred, since it may often prove difficult or impossible to obtain adequate fresh supplies of a particular plant whenever required.

4. *Availability at convenient periods of the year.* To avoid clashing with the seasonal operations of field spraying and recording, the laboratory tests have to be carried out as far as possible at other periods of the year. It is desirable, therefore, that large stocks of test insects should be obtainable at those periods when they can most conveniently be dealt with. This object can sometimes be attained by suitable adjustment of the temperature at which the insects are maintained.

5. *Suitability for use as dual purpose insects.* A species that can be used not only for ovicide but also for contact or stomach poison tests is economical of labour and equipment, since only one stock need be maintained for the several purposes.

6. *Economic importance.* The insects used should preferably be species that commonly occur as pests or are typical of common pests in their resistance to insecticides.

Whenever possible specially reared insects should be used, particularly for tests of contact insecticides and stomach poisons, since they are free from parasites and are more uniform in development than field-collected specimens. For tests of ovicides, field-collected eggs of certain species, e.g. *Aphis pomi* and *Oligonychus ulmi*, can well be employed, provided healthy parasite-free stocks can be obtained.

At East Malling, the ovicide tests have been carried out chiefly on eggs of the Vapourer Moth (*Orgyia antiqua* L.), the Winter Moth (*Operophtera brumata* L.), Green Apple Aphis (*Aphis pomi* de G.) and Red Spider (*Oligonychus ulmi* Koch).

VAPOURER MOTH.

The Vapourer Moth is a very useful test insect, since it is easily reared and handled and can be used as a dual purpose insect. The eggs, which are laid in convenient batches of two to three hundred, can be used for ovicide tests during the winter, whilst the larvae can be used for tests of contact insecticides and stomach poisons in the spring and summer. Under normal conditions the eggs are laid in August or September and hatch in the following May, but the periods during which eggs and larvae are available can be considerably lengthened. Thus, hatching can be delayed till August if the eggs are kept at a low temperature (5° C.) until a fortnight before the desired hatching time, whilst larvae can easily be obtained in December by incubating the eggs at 21° C.

Raising Stocks of Eggs.

An adequate stock of eggs can be raised within a year, from a nucleus of half a dozen wild batches which can be collected without much difficulty in almost any orchard. The larvae can be reared on foliage of Myrobolan, plum, apple, raspberry, privet and many other plants.

At East Malling the newly hatched larvae are kept in the laboratory in cages made up of hurricane-lamp chimneys and pots of sand (Pl. 2, Fig. 1). Shoots of Myrobolan are inserted in the sand, which has previously been wetted, and then enclosed by the lamp-glasses. About two hundred newly-hatched larvae are place in each cage, the top of which is then covered with a piece of muslin secured by an elastic band. For the very young larvae a much finer material such as organdie is advisable, but older larvae can easily be retained with a cheap grade of butter muslin. The cages are then arranged on shelves, the pots standing in saucers to which water can be added as required. The amount of water that must be added can be determined after a little experience—it must be sufficient to keep the foliage from wilting but not enough to cause undue condensation on the glass.

To avoid excessive mortality amongst the larvae, the food is renewed frequently. It can be kept in a fresh state for about a week, but is best changed after about four days. Whenever the food is changed the larvae are transferred to a fresh cage. This enables a count to be made and facilitates the removal of the larval excrement which would otherwise encourage the growth of moulds.

When the larvae have reached their second or third instar they are transferred to an outdoor insectary. There they are kept in a large cage, made of wood and wire gauze, and fed on Myrobolan shoots, the cut ends of which are thrust into wet sand on the floor of the cage.

When fully fed, the larvae will, if left undisturbed, crawl to the roof of the cage and spin up in the topmost corners, where large masses of cocoons will soon accumulate. To avoid this, the fully-fed larvae are removed to a " finishing cage ". There they are placed on a few shoots standing in sand and surrounded by four wooden boards. To the inner surfaces of the boards, paper folded into the shape of shelves is attached (Pl. 1, Fig. 2). Most of the larvae spin their cocoons on the paper shelves, and these can be removed before they become too full of cocoons and hung up in some convenient place until the moths emerge. The few larvae that reach the roof are allowed to pupate on strips of paper pinned to the framework of the cage.

After emergence the female moths remain on the outside of their cocoons, since they cannot fly, to await fertilization. The egg-batches are then laid on the cocoons or, occasionally, on the paper to which these are attached. Periodically, the batches are collected, graded, and stored in suitable containers. As there is no means of detecting infertile batches by the appearance of the eggs, it is advisable always to ascertain whether plenty of males are present, and to discard any batches that may have been laid in the absence of them.

Conducting the Tests.

The method adopted is based on one already used in 1926 and 1927 (2, 4), in that the principle is followed of setting aside a portion of each egg-batch to serve as a control and as a check on the fertility of the eggs. The present procedure differs greatly, however, from that formerly employed ; it is standardized, and enables quick results to be obtained, whilst the improved methods of rearing and handling the insects have resulted in a saving of labour and in greater fertility.

Only the best of the egg-batches are used for ovicide tests ; small batches and those containing collapsed or otherwise unsuitable eggs are discarded. Every batch is divided into four approximately equal portions, each of which can be dealt with separately and so enable as many as three insecticides to be tested on the same egg-batch. At least six replications, i.e. six batches comprising some fifteen hundred eggs, are used in a test.

Each quarter-batch is sprayed with the appropriate concentration of the material to be tested and placed in a small glass dish to drain. When dry it is placed in a small glass tube ($\frac{1}{4}$ inch diameter), the open end of which is then closed with a piece of organdie, held in place by a drilled cork or a piece of rubber tubing. A label bearing the egg-batch number (which enables the

history of the batch to be readily ascertained) and a letter representing the spray treatment is placed in the tube.

All the tubes of one test are packed in a shallow cardboard box, bearing the serial number of the test, and put into an incubator maintained at 21° C.

The compact arrangement of the tubes and the simple system of labelling enable the most to be made of limited incubator accommodation and facilitate handling and recording.

Hatching begins after 10-14 days—although eggs put in as early as November may take three weeks—and continues, usually, for 6-7 weeks. During the hatching period larvae are removed from the tubes every few days.

The eggs are counted with the aid of a binocular microscope at any time after hatching is finished. In addition to counts of hatched and unhatched eggs, it has been found convenient to record also the number of partly hatched eggs (i.e. those in which the larvae pierce the chorion but die before hatching properly), and of eggs collapsed or discoloured as a result of spray treatment.

A method of calculating the result of a test from the numbers of larvae that hatch out is often adopted to obtain an interim result on which to base further experimental work without having to wait for the final egg counts. The per cent. kill is reckoned as

$$100 - \frac{100 \times \text{(number of larvae hatched in sprayed series)}}{\text{number of larvae hatched in control series}}$$

This method is based on the assumption that the number of eggs used is approximately the same in each treatment, and gives results that agree very closely with those ultimately obtained from egg counts (Table I).

Laboratory ovicide trials with Vapourer Moth eggs stored under outdoor conditions can be undertaken during the months December to April inclusive, and, since an interim result can, as in the example quoted, be obtained within a month, as many as four consecutive interdependent series of tests can be carried out in a single season.

For treating the eggs with an insecticide a Tattersfield apparatus (5) is used. Such a machine is not essential, since satisfactory results can be obtained if the eggs are merely dipped in the insecticide. It was originally obtained for testing contact insecticides, but has since proved a quick and convenient means of applying insecticides to Vapourer Moth egg-batches, which can by its aid be dealt with at the rate of more than two a minute.

The apparatus is worked in a similar manner to that originally described by Tattersfield and Morris, but the dish of material to be treated is placed in a rectangular glass-sided box instead of in a cylindrical glass jar. An aperture in one side enables the spraying to be carried out rapidly and obviates repeated removal of the lid. The atomizer has been adequately described by Tattersfield (6). Two operators are needed. One prepares and applies the insecticides ;

TABLE I.

Showing the agreement between interim and final results in a laboratory test

(No. 0.70/35) on Vapourer Moth eggs.

Treatment.*	First interim result based on larval counts up to February 14th, 1936, i.e. two weeks after hatching began.		Second interim result based on larval counts up to April 6th, 1936, i.e. at end of hatching period.		Final result based on egg counts made on March 2nd, 1937.			
	No. of larvae hatched	% Reduction of hatch	No of larvae hatched †	% Reduction of hatch	Total No of eggs.	No. of eggs hatched.†	% eggs hatched.	% viable eggs killed.
A. 4% emulsion of a tar oil B.P. 250-300° C.	30	85·6	67	81·9	409	73	17·8	80·9
B. 4% ,, ,, ,, 300-350° C.	7	96·7	27	92·7	385	27	7·0	92·5
C. 4% ,, ,, ,, > 350° C.	65	68·9	150	59·6	377	151	40·1	56·9
D. Untreated Controls	209	—	371	—	401	373	93·0	—

* In this, as in other examples of laboratory tests given below, details of the composition of the insecticides used are omitted, since they are scarcely relevant to a discussion on biological methods, and will be fully dealt with elsewhere.

† The slight discrepancy between the figures in these two columns is accounted for by a few larvae hatching after April 6th.

the other prepares the eggs, puts them into the apparatus and removes them after they are sprayed. 2 c.c. of spray material is used to each dish of eggs, since, as Table II shows, this amount gives results comparable with those obtained when the eggs are dipped.

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TABLE II.

Showing the effect of differences in the method of applying an insecticide to Vapourer Moth eggs.

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Treatment.	% * hatch.	S.E.
A. Eggs sprayed with 1 c.c. of a 4% tar oil emulsion	14·1	4·7
B. ,, ,, 2 c.c. ,, ,, ,, ...	0·2 }	0·2
C. ,, dipped in a 4% tar oil emulsion	1·6 }	1·3
D. ,, Untreated Controls	82·5	7·1

    * In these Tables, brackets are used to indicate which differences are insignificant.

Winter Moth.

The Winter Moth possesses the obvious merit of being a common fruit pest. Unfortunately the larvae are somewhat difficult to rear and to handle in large numbers. Their cannibalist tendency often causes serious losses, and their habit of feeding enclosed in coiled-up or spun-together leaves makes re-feeding long and tedious.

Raising Stocks of Eggs.

It has been found best to raise stocks of eggs from moths collected on fruit trees in neglected plantations. Where such plantations are not available, it would be advisable to encourage the pest to develop on a small block of fruit trees by infesting them with larvae.

Being wingless, the female moth has to crawl up the trunk of the tree, from the soil wherein she pupated, to lay her eggs on the twigs. The males fly about at night and it is generally thought best to search for the ascending females after dusk. In a neglected plot of apple trees near the Research Station, however, it has never proved difficult to find sufficient moths during the daytime. Over 900 females were collected during the winter of 1936-37, and Fig. 1 shows that these were found during the period November 16th to January 8th, but chiefly during December. A less intensive search, carried out in the previous winter, yielded 130 moths, most of which were found between December 10th and 20th, a few being collected late in December and early in January, but none in November or after January 21st.

Attempts were also made to rear moths from larvae collected on fruit trees during the summer. Since many of the larvae were parasitized and others were

lost as a result of cannibalism, the pupae that developed were disappointingly few. About 150 pupae, reared in 1936, were kept in a cool laboratory, and moths emerged from 65% of them (72% when the pupae were stored in peat moss, and only 50% when they were stored in sand). 70% of the moths were females but, as these often began to lay before pairing could be effected, many of their eggs proved infertile. Emergence was spread over so long a period
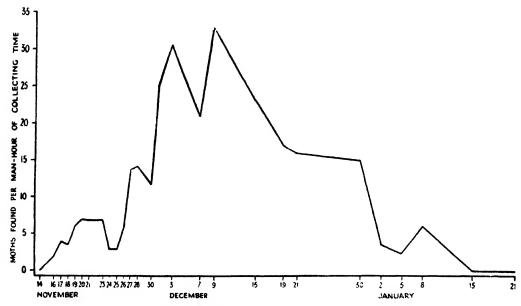

FIG. 1 *O. brumata* females collected in a neglected orchard in the winter of 1936-37.

(Fig. 2) that sufficient eggs for satisfactory ovicide tests could not be obtained at any one time.

Eggs, laid in sufficient numbers and on suitable material for ovicide tests, can be obtained by confining 15-20 female moths on apple shoots in a cage such as that illustrated (Pl. 2, Fig. 1). The eggs are laid on the shoots, mostly at the base of the buds, and also on the muslin cover of the lamp-glass. The
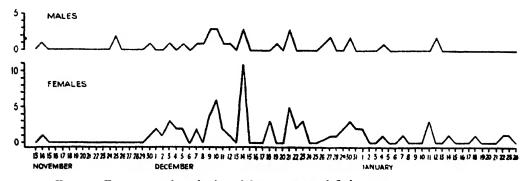

FIG. 2. Emergence of moths from laboratory-reared *O. brumata* pupae, 1936-37.

flower-pot portion of the cage stands in a saucer of water ; this keeps the sand moist and prevents the shoots shrivelling. Very little space is required, and as many as eight of these cages can, if necessary, be placed on a yard of ordinary nine-inch shelving.

The shoots should be clean and free from deep cracks or cankers and well furnished with very short spurs.   Satisfactory shoots have been obtained from trees of Lane's Prince Albert that were left unpruned for two seasons with the result that vigorous shoots grew out and later on developed plentiful fruit buds. Old shoots, with dead spurs or loose bark and crevices, should be avoided ; eggs are freely laid on them but are not easily found and counted.   On the other hand, moths cannot be induced to lay sufficient eggs on the current year's growths, which are smooth and bear only wood buds, even if cuts or scratches are first made in the bark.   Several attempts have been made to get them to lay on butchers' wooden skewers and other artificial substitutes for apple shoots, but without success.

Eggs can be obtained by confining moths in Petri dishes with discs of muslin on the bottoms, but, as so many of the eggs are laid on the glass and therefore of little use, such a method is wasteful.   Other receptacles have also been tried.   Large paper cylinders were useless, as eggs were seldom laid except in crevices formed by the overlapping edges of the paper.   When suspended in muslin bags the moths laid eggs readily, if somewhat unevenly, but soon perished.

A somewhat humid atmosphere seems to be essential to the moths and this is secured by confining them with twigs in lamp jars standing on moist sand.   Under these conditions wild females can be expected to live seven days and to produce 40-50 eggs apiece, of which less than 1% are infertile.   Bred females are usually more prolific but, even if males are provided, up to 25% of their eggs may be infertile.

Conducting the Tests.

Method 1. Shoots bearing sufficient eggs are selected and dipped, one at a time, in a glass cylinder containing the spray material to be tested.   They are then allowed to dry in the laboratory with their basal ends thrust into moist sand.   When dry they are placed in jars containing sand and water, suitably labelled (Pl. 2, Fig. 2), and placed in the insect-proof chambers of an insectary.   There they are lightly sprayed with water from time to time.   Five shoots are normally used for each treatment and can be relied on to bear 50-100 eggs each.

When hatching is finished, the shoots are brought into the laboratory and examined under a binocular microscope.   Hatched and unhatched eggs can readily be recognized, but, since many of them are thrust into crevices between

bud and stem or under loose bud scales, careful search is necessary to avoid overlooking some of the eggs. This search can be facilitated by first cutting the shoots up into one-inch lengths and then examining each piece separately. In counting, the total is best reckoned mentally and the number of either hatched or unhatched eggs recorded with a hand tally counter.

The hatched eggs are empty, colourless shells with a hole in one end ; the unhatched are either reddish brown or black in colour and may be partly collapsed. If counting is long delayed the contents of some of the unhatched eggs may shrink and become inconspicuous. Care is then needed to distinguish between hatched eggs and such eggs as these, since both have colourless shells.

The result of part of a typical ovicide test is shown in Table III.

TABLE III.

Example of laboratory test (No. 0.14/36) *on Winter Moth eggs.*

Treatment	Total No. of eggs	% hatched.	S.E.
A. Petroleum oil, 4% emulsion ..	461	0·3	0·3
G. Tar oil 4% ,, ..	352	8·6	4·1
B. Petroleum oil, 2%	333	20·4	5·0
H. Tar oil 2%	516	23·2	1·9
J. Soap Controls	513	84·5	3·6
K. Untreated Controls	367	92·8	1·4

Method 2. Whenever the lamp-glass method of obtaining eggs is employed some eggs are laid on the muslin covers.* Pieces of this muslin, bearing sufficient eggs, are dipped in the insecticide, hung up to dry and placed in $3'' \times 1''$ glass tubes, the corks of which are drilled and covered with muslin. These are then placed in an incubator or stored in the laboratory. As the larvae hatch out they are removed and counted, thereby obviating the necessity of counting the hatched eggs later on. The eggs remaining unhatched can best be counted by placing the muslin between two sheets of glass, one of which is marked in centimetre squares in the manner of the Vyvyan Integrator (7). The glass plates hold the muslin flat, and the centimetre divisions facilitate counting.

Eggs laid on apple shoots can also be dealt with in much the same way, provided the shoots are cut into pieces small enough to be placed in glass tubes. Shoots are not entirely satisfactory, however, since they are apt to become mouldy in the incubator. A further objection is that the larvae must be removed and counted almost daily during the hatching period, otherwise

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* If eggs laid on shoots only are required, this wastage can be avoided by using cellophane in place of muslin as a means of closing the open ends of the lamp-glasses.

they soon die and shrivel, and so may be confused with small particles of dirt that flake off the twigs.

The muslin method is useful, particularly when quick results are desired, since the device, adopted in the Vapourer Moth ovicide tests, of calculating a result from the numbers of larvae that hatch, can be used. On the other hand, it may not prove as reliable as Method 1, since the muslin is liable to absorb some of the insecticide and so retain it in close contact with the eggs.

Results obtained with the two methods are compared in Table IV. Method 2 gave a lower hatch of treated but a slightly greater hatch of untreated eggs, both differences being significant. Further information on the relative merits of the two methods is being sought.

TABLE IV.

Comparison of two methods of conducting insecticide tests on Winter Moth eggs.

Treatment.	Mean % hatch.		S.E. of difference.	Sig. Diff.
	Method 1.	*Method 2.*		
Petroleum oil, 2% emulsion ..	20·4	1·5	5·04	11·6
Untreated Control	92·8	98·8	1·66	3·8

GREEN APPLE APHIS.

The Green Apple Aphis feeds and lays its eggs clustered closely together on the current season's growths. It is a convenient species for ovicide tests and, for them, it may safely be regarded as similar in behaviour to other aphides that overwinter as eggs on fruit trees and bushes.

Collection of Egg Material.

Supplies of eggs, which are laid in October, November and December, can most conveniently be obtained from apple rootstock stool-beds. If such beds are maintained solely for this purpose, the stocks should not be cut till January, since aphides persist and eggs are laid up to the end of December. At East Malling, the rootstocks are normally lifted in November and December, and prepared for sale. Aphis infested shoots required for laboratory tests have, therefore, to be cut out in November. Since these are not required for use till January they are heeled-in in an enclosure protected from sparrows and other birds by walls and roof of ½" mesh galvanized wire netting. Well infested stocks are chosen, the soil is removed from the base and the stems are cut below soil level leaving some roots attached. Great care is needed in lifting

and transporting the stocks, but, if they are grasped only by the roots, they can be handled without damage either to the aphides or the eggs.

Good colonies of eggs have been found in stools of Malling Types II, XVI and XII, each of which produces long straight growths, convenient to handle and well suited to the procedure followed in carrying out the tests. Shoots of Types I and IX have usually had to be rejected on account of poor infestation or shortness of growth.

The long vigorous shoots that are produced by heavily pruned trees are liable to attack by *A. pomi*, and will often provide suitable egg material. Stool-beds, however, are more reliable as a source of supply.

Conduct of the Tests.

The ingenious method described by Kearns and Martin (3) is now used for all toxicity trials on *A. pomi* eggs. The procedure has been somewhat modified to suit East Malling facilities and to bring it into line with that followed with other eggs, such as those of Winter Moth and Red Spider. Formerly, the results of tests carried out on *A. pomi* eggs could be assessed only by counting the hatched and unhatched eggs that could be seen in limited fields of view under a binocular microscope, since they are so closely crowded together as to make it impossible to count all the eggs on a twig. Now, however, all the eggs can be removed from the twigs and counted with reasonable accuracy.

When all preparations have been made for carrying out a test, the heeled-in stocks are cut (as near the base as possible) with secateurs and brought into the laboratory. They are handled by their basal ends which usually bear very few eggs and are in any case always discarded. In the laboratory they are carefully examined and the best of them cut into ten-inch lengths. Shoots bearing collapsed eggs and those portions that are cankered or killed by aphis attack are discarded.

The pieces of shoot are dipped, one at a time, in a 250 c.c. graduated cylinder filled to the brim with 300 c.c. of spray fluid. Thorough wetting is ensured by dipping, lifting and redipping each shoot and then repeating the same movements with the shoot reversed. The total immersion time is about ten seconds. The shoots, five of which are used for each treatment, need be handled only by the ends, these being discarded when the final sampling is carried out.

After being dipped, the shoots stand in the laboratory to dry, with their basal ends thrust into moist sand in a wooden box. They are then transferred to jars of wet sand (Pl. 2, Fig. 2). The five shoots of one treatment easily fit into one jar, which has the appropriate test number and treatment letter painted on it. A large number of such jars can be stored in a small insect-proof compartment of the insectary. Water is added to the jars as often as is necessary and occasionally the shoots themselves are lightly sprayed with water.

All tests, with the exception of special time of treatment trials, are carried out in January and February, and the above method of dipping and handling has proved simple, rapid and convenient.

The eggs begin to hatch at the end of March. Just before this happens small samples are cut from the twigs and placed in $4\frac{1}{2}'' \times 2''$ glass tubes (Pl. 1, Fig. 1). These stand on shelves in a cool laboratory until all the aphides that hatch out are dead.

TABLE V.

Example of laboratory test (No. 0.8/36) *on* A. pomi *eggs*.

Treatment.	Twig No.	No. of eggs used.	% hatch.	Mean % hatch.	S.E.
C. Nicotine 0·1% + Soap	1	322	0		
	2	112	0		
	3	287	0	0	0
	4	206	0		
	5	137	0		
D. Nicotine 0·07% + Soap ..	1	470	20·6		
	2	321	21·5		
	3	104	18·3	20·4	0·6
	4	97	21·6		
	5	192	19·8		
E. Nicotine 0·04% + Soap ..	1	203	38·4		
	2	285	30·2		
	3	172	29·1	32·8	1·6
	4	86	33·7		
B. Soap Controls	1	344	97·4		
	2	383	95·0		
	3	186	75·3	88·4	3·8
	4	410	88·5		
	5	120	85·8		
F. Untreated Controls	1	235	94·5		
	2	232	94·0		
	3	820	95·7	94·2	0·5
	4	291	94·2		
	5	68	92·6		

It has been found that mould readily develops on shoots kept in tightly corked tubes. If, however, a large hole is drilled through each cork the shoots dry out without harm to the eggs. The aphides are prevented from escaping by a piece of fine muslin or organdie held in place by the cork. Again, in cutting the samples, it is better to cut off portions that can be seen to bear about 300 eggs, which may be anything from one to four inches long, rather than to cut standard lengths of, say, two inches entirely at random. Excessive variation in the numbers of eggs used is thereby avoided.

To remove the eggs from the shoots, these are warmed in a test tube containing 10% caustic potash for ten minutes, the fluid poured off and filtered ; further warm caustic potash is added and poured off in a similar manner until eggs can no longer be removed.   The shoot is finally shaken vigorously in a test tube of water to determine whether any eggs remain.   The filtrate can be used several times.   The eggs are washed off the filter papers with water and counted in a Petri dish standing on squared paper.   The dead aphides are shaken out of the tubes and counted to determine the number of eggs that hatched.

The result of part of a typical test is shown in Table V.

RED SPIDER.

Although in many ways unsatisfactory for laboratory use, Red Spider has some value as being an example of that small group of pests susceptible to petroleum oil but unaffected by tar oil sprays.

Source of Eggs.

The winter eggs are laid on twigs and branches of apple, plum and other fruit trees.   They are rarely laid on the current year's wood, so that the older shoots and spurs form the only material that can well be used in laboratory tests.   There is little difficulty in obtaining adequate supplies of eggs since, in most districts, Red Spider rapidly increases on trees that are not sprayed regularly with lime-sulphur, and particularly on trees that are annually sprayed with a tar oil winter wash.

Shoots bearing eggs can be cut and kept in the insectary or in the laboratory with the cut ends immersed in wet sand or water.   Provided the shoots are moistened from time to time by spraying them with water, the eggs hatch satisfactorily.

Conduct of the Tests.

Method 1.   Shoots bearing eggs are dipped into the spray fluid to be tested, drained and stood in jars of sand and water on a bench to await hatching. When hatching is complete, counts are made, with the aid of a binocular microscope and a mechanical hand counter, of hatched and unhatched eggs, on short lengths of shoot chosen at random.   In almost any sample of Red Spider eggs, some shells of previous seasons' eggs can be found, still attached to the bark. As it is usually impossible to differentiate between these old shells and those of newly hatched eggs, the counts are not entirely satisfactory as an index of the degree of control.   The number of old shells present has relatively more effect on results obtained with sprays that give a high kill than on those obtained with sprays of indifferent efficiency.

One method of overcoming this difficulty is to estimate the proportion of old shells in the egg population by adequate counts of random samples, made before the beginning of hatching. The actual percentage hatch is then reckoned as $\dfrac{100\,(H-S)}{100-S}$, where H is the apparent per cent. hatch and S is the estimated percentage of old shells present. Thus, supposing egg counts indicate apparent hatches of 24% and 92% on sprayed and unsprayed shoots, and that the proportion of old shells has previously been estimated at 20%, then the actual hatch in the two series would be $\dfrac{100\,(24-20)}{100-20}$ % and $\dfrac{100\,(92-20)}{100-20}$ %, i.e. 5%, and 90%, respectively. Frequently, however, the proportion of old shells varies so greatly from shoot to shoot that it can be estimated only within very wide limits, when the correction cannot well be applied.

Data obtained by this method in a small scale test on Red Spider eggs are summarized in Table VI.

TABLE VI.

Example of laboratory test (No. 0.97/35) *on Red Spider eggs. Method 1.*

Treatment.	No. of eggs used.	% hatch	S E.	Calculated "actual" hatch, %
A. Petroleum oil, 6% emulsion ..	580	30·1 ⎫	2·8	0
B. ,, ,, 4% ,, ..	400	33·2 ⎬	3·0	4
C. ,, ,, 2% ,, ..	400	30·0 ⎭	4·9	0
D. Tar oil 6% ,, ..	400	68·0	7·4	54
K. Untreated Controls	330	89·5	2·5	85

Although counts of random samples of eggs indicated a moderately high apparent hatch in A, B and C, no mites were observed to hatch in A and very few indeed in B or C. It is evident, therefore, that some 30% of the eggs were old shells. Using this figure to calculate the actual hatch, the result shown in column 5 is obtained. This agrees closely with notes made at hatching time on the degree of control given by the various treatments, but the uncorrected data (columns 3 and 4) are, in this example, adequate for tests of significance.

Method 2. As the above method is in many ways unsatisfactory, a modification of the procedure has been devised but has not yet been thoroughly tried out. From the shoots used in a test, random samples ½″ to 1″ long are cut off and placed in glass tubes fitted with drilled corks. The mites hatch out but are prevented from leaving the tubes by a piece of closely woven material such as calico, which, however, allows sufficient ventilation to prevent mould

growing on the cut shoots. After hatching, the mites can be counted, with some difficulty, and the unhatched eggs can be removed from the shoots with caustic potash and counted in the manner adopted for *A. pomi* eggs. The old shells are not counted and are thus of no consequence.

An experiment designed to compare the above two methods of testing ovicides on *O. ulmi* was undertaken in 1937. This failed, since, for some unknown reason, hardly any of the eggs hatched, even on the trees from which the shoots were taken.

Carroll (1) has described a method of conducting laboratory trials on Red Spider eggs. This, however, necessitates much tedious counting of mites and eggs and the resulting data are not altogether convenient for statistical treatment.

TESTING FOR SPRAY DAMAGE.

Before new or little known insecticides are used in field trials it is important that something should be known of their phytocidal properties.

The more promising of the materials undergoing tests in the laboratory are, therefore, tried out at suitable concentrations on established fruit trees and bushes. It is convenient to use a single large branch of a tree as a unit. Several sprays can then be tested on the same tree, thereby avoiding the necessity of using (and possibly destroying) a large block of valuable trees.

Selected branches are sprayed by means of a knapsack machine or, if the amount of spray material available is small, with a small double-action hand-pump sprayer. Care is taken to wet all parts of the branch thoroughly, and the spray is prevented from reaching the rest of the tree by large pieces of sacking hung over the neighbouring branches.

Ovicides are tested at both dormant and semi-dormant stages in the development of the tree, and, at the time of spraying, notes are made on such incidental matters as ease of mixing, wetting power and rate of drying of the various sprays. After spraying, periodical observations are made on the effect on the development of the buds. These tests are carried out chiefly on Lord Derby, Lane's Prince Albert and Cox's Orange Pippin apples, and on Victoria, Pond's Seedling, Monarch and Czar plums.

Materials that cause injury at concentrations high enough to kill insect eggs are usually not further considered. Those that cause no injury are used, first in small-scale then in larger, field trials where, in addition to their effect on particular pests, any tendency to cause injury to the trees is noted. No new material can be regarded as safe for use in commercial orchards until it has undergone field trials for several seasons, since differences of season, soil, variety and rootstock often have a great, though little understood, influence on the propensity of fruit trees to suffer spray injury.

The single branch system can seldom be used on bushes and never on such plants as strawberries. For phytocide tests on these, individual plants or sets of plants must be used as units, and the effect of any normal variation in susceptibility provided for by adequate replication of the treatments.

SUMMARY.

Standardized methods of conducting biological tests with suitable insect species as indicators are indispensable for the rapid sorting of new and potential insecticides and for the correlation of toxicity and chemical constitution. Careful attention to details of rearing and handling is necessary to eliminate all but inherent sources of variation in the insects used.

For testing ovicides the eggs of *Orgyia antiqua* L., *Operophtera brumata* L., *Aphis pomi* de G. and, to a lesser extent, *Oligonychus ulmi* Koch, are used.

O. antiqua is bred in large numbers and is useful for tests of contact and stomach poisons in addition to ovicides. After insecticidal treatment the eggs are incubated at 21° C. to accelerate hatching and to get early results.

Females of *O. brumata* are collected in orchards and induced to lay eggs on twigs in special cages. The twigs are then dipped in the insecticide and the result assessed from subsequent counts of hatched and unhatched eggs. Eggs laid on muslin can also be utilized if the testing procedure is suitably modified.

Shoots bearing eggs of *A. pomi* are collected from apple rootstock stool-beds and treated by an adaption of the Kearns and Martin method.

O. ulmi eggs can be used but are not altogether satisfactory.

Spray materials that show sufficient promise in laboratory tests are applied to branches of fruit trees to ascertain whether they are safe to use. If satisfactory, they then undergo successive stages of field trial.

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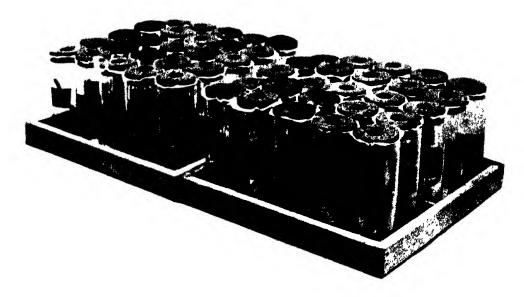
PLATE I

FIG. 1. Shoot samples in an *L. pomi* ovicide test. Aphides can be seen in some of the tubes.
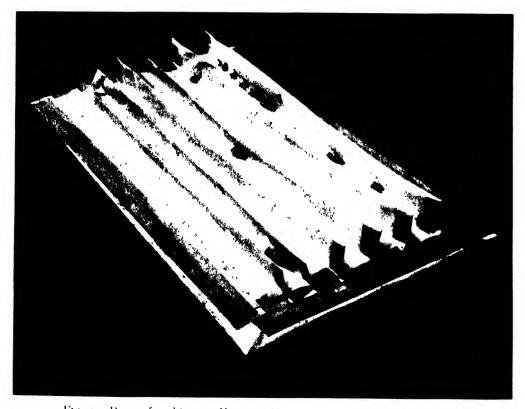

FIG. 2. Device for obtaining Vapourer Moth cocoons in a convenient form.

PLATE II

FIG. 1 Cage used for oviposition by Winter Moth (*Operophtera brumata* L.) females. Similar cages are used to confine small Vapourer Moth larvae on Myrobolan foliage.

FIG. 2 Twigs bearing eggs of *Aphis pomi* de G that have been dipped in an insecticide, ready for removal to the insectary. A similar arrangement is adopted for twigs bearing eggs of Winter Moth and of Red Spider.

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BOOK REVIEWS

THE DESIGN OF EXPERIMENTS. By R. A. FISHER, Sc.D., F.R.S. (Edinburgh and London: Oliver & Boyd. Second Edition. 1937. Price 12s. 6d. net.)

Since Dr. Fisher's first book appeared twelve years ago, a vast amount of interest has grown up concerning the design and interpretation of experiments. During this time a volume of literature on this subject has accumulated, which has greatly outgrown the single chapter devoted to it in that book; so much so that an authoritative book from the originator, devoted exclusively to the application of modern statistical ideas to the conduct and interpretation of experiments, was badly needed. In 1935 this need was met by the publication of the present book, the second edition of which has now appeared.

To say that every research worker would do well to study the opening chapters of this book, whether he be at the start of his career, or in a position with many years of research behind him, is hardly an overstatement. Too often experimental designs betray muddled thinking and a lack of appreciation of the fundamental principles which underlie all experiments, even though the methods of experimentation may be good ones. In horticulture this is especially true, for it has happened all too frequently in the past that a lack of clear thinking in the preliminary stages of a long term field trial has led to years of wasted energy and, in the end, to meaningless results.

This book should go far to place matters on a sounder footing, for in it are set forth clearly and logically the principles of experimental design, showing how once a problem is formulated, an experiment may be planned so as to answer, with any desired precision, definite questions propounded in order to throw light upon it.

Having described, the fundamental ideas behind all experiments the author goes on to develop and explain the various methods of arranging an experiment, associated with his name, and now in common use, ranging from simple randomized blocks and Latin squares to much more complex designs embodying the complete or partial confounding of minor issues in the trial. These latter are built up on the basis of factorial design, the principles of which are explained in Chapter VI.

Here a word of warning should be said, for, while to the inexperienced some of these more complex arrangements are extremely fascinating, they are to be used with the utmost care and circumspection. It is of little use to take a razor to fell a tree ; and there are as yet few horticultural crops in which the material available for experiment is sufficiently standardized for these refined tools to be used without discretion

This is not to say that these methods of experimentation are not exceedingly valuable in their proper sphere ; but, for the most part, the horticulturist would do better to adhere to the simpler forms of plot arrangement, such as randomized blocks, until such a time as he is looking for much finer differences between the treatments in his experiment than are usual to-day.

The book concludes with a discussion of tests of significance and precision and the measurement of amount of information in general. No doubt it will become in time the vade-mecum of practical experimenters, perhaps to an even greater extent than its forerunner, which is already to be found on the desks of research workers all over the world.   T.N.H.

COMMERCIAL APPLE GROWING. By A. H. HOARE. (Martin Hopkinson Ltd., London, 1937, 237 pp. Price 10s. net.)

This book deals exclusively with the growing, packing and marketing of apples on a commercial scale in this country.

On the hypothesis that of the apples consumed in the raw state in the United Kingdom only one-quarter are at present produced by the home grower, the author considers that there is ample scope for the production of high grade dessert apples here. He urges growers to aim at producing 70 per cent. of combined extra fancy and fancy grades, and recommends a production unit large enough to maintain a minimum output of 50 bushels a day during the marketing season, of not more than four good varieties. The main commercial varieties are briefly described and sound advice is given on planning and planting. Seven years are regarded as the period which may be expected to elapse before large bush trees on stocks of medium vigour can be expected to show any substantial returns. Pruning, manuring and soil management are adequately dealt with in separate chapters. The author has some interesting things to say on certain other factors affecting tree performance, such as June drop, bark ringing, biennial bearing and fruit thinning, but he is careful to point out that these matters are still under investigation.

Full details are given of a modern form of orchard heater, and packing, marketing and storage methods are described accurately and with a marked economy of words. The chapters on the control of pests and diseases contain an excellent description of spraying technique and provide some interesting costing figures. The author maintains that a grower should be in a position to spray efficiently the whole of his acreage in from 3-5 days. The section on finance and accounts and the author's personal note at the end of the book deserve careful study by every prospective fruitgrower. An appendix contains a spray calendar and some facts and figures which should prove useful for reference. This is a valuable textbook about a specialized industry ; it is written for business men by a specialist who knows what he is writing about and who has made full use of the findings of science and practice.   N.B.B.